Vol. 9 No. 2

February, 1943

THE BOTANICAL REVIEW

Interpreting Botanical Progress

CONTENTS

PROPOSED FUTURE
CONTENTS ON REAR COVER

Published Monthly except August and September at
North Queen St. and McGovern Ave., Lancaster, Pa.

Entered as second-class mail matter February 4, 1935, at the Post Office at
Lancaster, Pennsylvania, under the Act of March 3, 1879.

THE BOTANICAL REVIEW

Founded in 1935 by
H. A. GLEASON AND E. H. FULLING
Managed, edited and published at
The New York Botanical Garden
by
E. H. FULLING

THE BOTANICAL REVIEW is published monthly except August and September. Subscription rate for the United States is $4.00 per year; for Canada and other foreign countries $4.25. All subscriptions are payable in advance at par in Lancaster, Pa., or New York, and will be accepted for complete volumes only. Checks should be made payable to THE BOTANICAL REVIEW.

Manuscripts are prepared by contributing authors only upon invitation and on the suggestion or with the approval of the advisory editor in whose field the subject lies. Unsolicited manuscripts will not be considered. The editors, nevertheless, welcome suggestions of topics which might properly be reviewed.

Missing numbers can not be supplied unless notice is received within two months after appearance of issues concerned.

All correspondence should be addressed to THE BOTANICAL REVIEW, at N. Queen St. and McGovern Ave., Lancaster, Pa., or Fordham P. O., New York, N. Y.

THE BOTANICAL REVIEW

VOL. IX FEBRUARY, 1943 No. 2

PROTOPLASMIC STREAMING

WILLIAM SEIFRIZ
University of Pennsylvania

CONTENTS

I. INTRODUCTION

1. Discovery.

Protoplasmic streaming was observed before the existence of protoplasm as such was known. Corti (48, 49) is generally credited with being the first to note the motion of protoplasm, in 1772, but he thought the moving substance was cell sap; he showed, however, that it stopped at death. That inimitable observer, Rösel von Rosenhof, described the movement of *Amoeba* in 1755. It does not seem likely that he was wholly unaware of the fact that what he saw was living matter, for that is about all there is to an amoeba.

Corti misinterpreted his own observations because of the influence of Harvey's discovery of the circulation of blood, in 1628, which led to speculations on the probable occurrence of a similar process in plants. Corti later corrected his error. This done, he concluded that streaming is a normal process and probably occurs in every living cell.

No complete understanding of the significance of protoplasmic streaming could be attained until the fact that protoplasm is the living substance was fully appreciated. This did not come until 1835. Three years later, Dutrochet (56) still referred to the circulation of the "fluid" in *Chara*.

Corti's observation was forgotten and it remained for Treviranus (197, 198) in 1807 to rediscover protoplasmic streaming in *Chara*. The observation was questioned by Martius and others until Amici (3, 4, 5, 6) finally convinced every one of its reality.

The studies of Amici aroused widespread attention and were the incentive which gave rise to the discovery of protoplasmic streaming in *Vallisneria* and *Hydrocharis* by Meyen (129) in 1827, and in *Tradescantia* by Robert Brown (26) in 1831. Meyen (130) in 1838 presented, in his "Pflanzenphysiologie", an excellent resumé of what was then known, to which he added many observations of his own. A review of early work and hypotheses on protoplasmic streaming is given by Goeppert and Cohn (76).

During the 40 years intervening between the experiments of the physicist Amici (3, 4) in 1820, and the first complete expression of the true role of the living substance by Schultze in 1861 (164), there took place some of the best and most fundamental work on protoplasm ever done. These were the years which produced the pioneer leaders in modern biology: Dujardin (55) who gave the first accurate description of protoplasm; von Mohl (133) who in 1846 reestablished the word "protoplasm", first introduced by Purkinje six years earlier; Naegeli (134) with his studies on protoplasmic structure; de Bary (10, 11) who discovered streaming in the plasmodia of Myxomycetes; and Schultze (163, 164, 165) who described the movements of the granular plasm in the pseudopodia of *Amoeba* and the Foraminifera, and, in 1861, rounded out the whole story with the first full recognition of the cell as an organized living unit.

From the foregoing list I have omitted the names of two men who

had a profound influence on cellular physiology, Schleiden (160) and Schwann (168). They are generally regarded as the authors of the cell theory. Guilliermond (78) in 1940 still gave them credit for it. But Americans, since 1914, have buffeted the authorship of the cell theory from country to country in a futile attempt to keep up with world politics. Actually, there was no one discoverer of the cell theory. It was not, in the first place, a discovery, but a development in the minds of scientists at work in the early years of the last century, and at least eight men working independently presented the theory at the same time.

Work on protoplasmic streaming has been active over the past century. During this period two thorough works on the subject have appeared, the monograph of Ewart (59) and an article by Vouk (209). Renewed activity has recently come about through introduction of slime mold culture into laboratories. Myxomycetes are particularly fine material for study of protoplasmic streaming, for the flow is continuous and exhibits a remarkable rhythmical shuttle movement. The laboratory culturing of slime molds was developed by several people, among whom Howard (91) and Camp (34) have played a prominent part.

All in all, the future of research on protoplasmic streaming is bright. To single out any biological process as more significant than another when all are necessary is impossible, yet surely none reveals the character of protoplasm more than its motion. Long before photosynthesis and reproduction had become symbols of life, the capacity to move on the part of that primordial droplet of ooze must have been the first visible indication that life on earth had arrived.

2. Prevalence.

A student told to search for protoplasmic streaming would, should he select living cells at random, report that the phenomenon is an exceptional one, for rare is the cell in which visible protoplasmic movement is always present. To this statement I hasten to add another, that rare is the cell, if indeed such exists, in which protoplasmic movement does not at some time take place. In a general way, cells may be put into one of three classes as regards streaming: those in which streaming is always present, as in Myxomycetes; occasionally present, as in *Elodea;* and those in which streaming

is never very active, as in some parenchymatous tissue. The absence of protoplasmic streaming, in the sense of no flow in a definite current, does not mean that the protoplasm is without motion. There is no living cell in which the protoplasm is wholly at rest at all times. The protoplasm in the young cells of primary meristems shows irregular movements which later, as vacuoles are formed, become established as circulatory streaming, or as rotational flow.

Protoplasmic movement is not always in the form of what is commonly called streaming. It is often a restricted turbulent motion. Local currents are common in physiologically active cells. Pfeffer (144) pointed out that temporary local movement exists in young cells which are acquiring the full power of streaming, and Naegeli (135) said the same was true when streaming is resumed in anesthetized cells. However, protoplasmic movement in the form of streaming is not uncommon. The protoplasm of some organisms is always in active flow, notably that of *Chara, Nitella* and the Myxomycetes.

Bushee (33) gives a long list of greenhouse plants which exhibit streaming. He recommends particularly the hairs of *Gloxinia speciosa.* How long and varied is the list of plants in which streaming is of frequent if not constant occurrence is indicated by the brief table of streaming rates given in Section IV–2.

Active flow commonly occurs in *Elodea* leaf cells, *Trianaea* root hairs, staminal hairs of *Tradescantia* and cucurbits, the stinging hairs of *Urtica,* the hyphae of pollen tubes, fern prothallia, *Vaucheria, etc.*

II. FORMS OF PROTOPLASMIC MOVEMENT

1. Streaming.

Among the many forms of protoplasmic movement, I shall, perhaps arbitrarily, limit streaming to that activity within cells which is a general flow of the entire protoplasmic contents in a definite current. To be sure, most forms of protoplasmic movement are associated with currents or lines of flow, but to include all under streaming would be more arbitrary than would be the exclusion of some of them. It is particularly movements of cells and tissues as a whole which I wish to exclude from protoplasmic streaming. Certain of them, such as muscular activity, are quite obviously not

protoplasmic streaming, though they may involve it. Others, such as amoeboid movement, are more closely comparable to streaming and are by some investigators so regarded.

Were amoeboid movement and protoplasmic streaming one and the same phenomenon, then it would be my duty to review the literature on amoeboid movement, which would be a tremendous task. This might be sufficient reason for me to distinguish between the two, but there are other grounds for regarding them as separate phenomena. Were they indissociable, then where one occurs the other would of necessity also be present, but this is not true. It would be a great stretch of the usual connotation of amoeboid movement to call the circulatory streaming of protoplasm in a closed plant cell by that name, and the reverse is equally true. An analogy will help make this clear. A tractor with a caterpillar tread advances because of the rotation of the tread, but the rotation of the tread is not the same as the advance of the tractor.

I shall, therefore, restrict streaming to the flow of protoplasm in definite currents within the cell, and not regard locomotion of the cell as a whole, or change in body form, as streaming, but only as possible functions of streaming.

The attempt is sometimes made in discussions on protoplasmic movement to distinguish between active and passive streaming. This distinction is occasionally justified, but often unnecessary and sometimes futile. The movement of a protozoan is active and that of an oil droplet in a protoplasmic stream is passive; this is obvious. But a distinction between active and passive streaming should not be too arbitrarily made until the mechanism of protoplasmic flow is better known. At present, how this is viewed depends in large measure on the convictions of the observer (see Section XII).

2. Amoeboid movement.

I have objected to the view which holds that amoeboid movement and protoplasmic streaming are one and the same phenomenon. To this conclusion a number of able workers on protoplasmic streaming will object. They will hold that cyclosis is fundamentally related to amoeboid movement. Related, yes, just as the rotation of a caterpillar tread is related to the forward motion of the tractor, but they are not one and the same thing.

Camp (35) regards protoplasmic streaming and locomotion in

slime molds as similar to the movement of rhizopods, and concludes that when the correct explanation is found for the one, it will hold for the other. In a limited sense, this is a permissible deduction, but if it is carried further, as may well be done, to include ciliary motion and irritability, one arrives at a very broad interpretation of protoplasmic streaming indeed.

The opponents of the view which I hold would probably say that amoeboid movement consists of two motions: *a*) change in outline of the body, of the ectoplasm; and *b*) inner protoplasmic movements, *i.e.*, streaming of the endoplasm. It is true that inner protoplasmic movement is probably responsible for pseudopodium formation, but it is also true that change in body form, which is the essential thing in amoeboid movement, can take place without an inner protoplasmic flow, *e.g.*, in muscular activity.

Hilton (84) states that a plasmodium has two characteristic movements, a streaming movement of the interior and more liquid plasma, and an amoeboid movement by which the whole mass changes its form and travels about. He concludes that the two processes are so closely related that a description of the one necessarily involves allusion to the other, but they are sufficiently distinct for separate consideration.

There is good reason to group all forms of protoplasmic motion into one great class, for the basic mechanism may be the same in all; that is to say, protoplasmic streaming, amoeboid movement, ciliary motion, muscular activity, *etc.*, may all involve one fundamental type of mechanism (see Section XII–11).

3. Euglenoid movement.

Euglenoid movement is defined by Küster (114) as the motion displayed by naked cells when they round up, stretch lengthwise, become sharply constricted in the center, and go through other similar motions, but always return to their original form. The movement is also termed "Metabolie".

Pascher (142) observed peculiar euglenoid movements in *Medusochloris*. Kamiya (99) noticed that euglenoid movement is characterized by a regular rhythm when the organism is transferred to a solution of greater acidity or alkalinity. The hydrogen ion has, therefore, come to be regarded as the stimulating factor, but Alexander (1) says that the response is quite evidently associated

with any sudden change in environment, rather than with the exact nature of the new environment.

This interesting form of protoplasmic motion resembles peristalsis and is another example of a change in body form which need not involve protoplasmic streaming, yet the two may be associated.

Klebs (108) accomplished fundamental work on metaboly; he gives an extensive bibliography.

4. Ciliary movement.

Propulsion of cells by ciliary movement is an example of cellular locomotion not necessarily involving protoplasmic streaming. It has been suggested that ciliary activity involves protoplasmic flow (the theory is discussed in Section II–6–F). There is, however, as yet no adequate evidence for it. Ciliary motion is probably a case of protoplasmic contractility (see Section XII–11).

5. Sliding and gliding movements.

Motility of bacteria, blue-green algae, desmids and diatoms has long been a subject of discussion quite apart from protoplasmic streaming, though the latter may be responsible in some cases. Locomotion of certain lower organisms has been ascribed to a moving protoplasmic thread running parallel to and outside the cell. It leaves the cell at the forward end and enters it at the rear. The mechanism is comparable to that of a caterpillar tractor. Hypotheses of this kind have their minor variations; thus, Verworn (204) proposed a secretory mechanism in which the flowing of an "outer mucus" serves to propel the cell. The outer mucous material of *Oscillatoria* was regarded as a modified plasma membrane. It was also stated that streaming within *Oscillatoria* occurred only when the algal filament is in motion, thus implying that the latter is responsible for the streaming.

Slightly different is the hypothesis of Prell (149) who thought that spiral strands of jelly are extruded from the pores of desmids, and that their screw-like motion causes movement of the cell.

There was also an osmotic theory to account for the sliding and gliding movements of blue-green algae, desmids and diatoms. The hypothesis received considerable support, though it rests principally on assumption. It was assumed that movement is due to diosmotic forces resulting from a turgor gradient along the filament. Vines

(207) thought creeping movements suggestive of pseudopodial action, but that swimming lends support to an osmotic theory. Burkholder (32) reviews the subject and concludes that none of the mechanisms—growth, cilia, osmotic currents, excretions, surface tension or peristaltic contractility—is satisfactory. Burkholder's rather pessimistic statements apply to most biological theories, but at that they are interesting and some undoubtedly closely approach the actual truth.

Höfler (86) recently reviewed the problem of the movement of diatoms.

6. Independent motion of cell parts.

A. *Granules.*

There has long prevailed among biologists the belief that certain cell inclusions possess independent motility. Without wholly denying this, it can nevertheless be said with certainty that when protoplasm flows, the cytoplasm flows and not the particles alone. (In this connection see Section V.)

The cell structures to which an independent motion has most often been attributed are mitochondrial granules, chloroplasts and nuclei. Cell granules were once regarded as colonies of bacteria, capable of independent motility. Altmann (2) postulated not only an independent motion but a wholly autonomous existence of the granules. He thought the granules living units, morphologically and physiologically independent of each other and of the cell which houses them. He called them "bioplasts". This view is no longer held. To be sure, all living parts of a cell lead a somewhat independent existence; thus, the chloroplasts grow and reproduce quite aside from activities of the cell as a whole, and the nucleus is quasi-independent; but neither is an independent organism, nor does it appear that either has a form of automotility.

B. *Chloroplasts.*

There still persists some evidence, or possibly it too is only a belief, that chloroplasts undergo a motion of their own. If true, the movement is amoeboid. Independent amoeboid movement of chloroplasts was postulated to account for their photostatic migration in the cell, but it appears more likely that the chloroplasts are carried by the protoplasm.

So firmly established did the idea that chloroplasts possess independent motility become that some novel suggestions arose as to the mechanism. Hörmann (89) postulated a layer of motory protoplasm.

C. *Nuclei, nucleoli and mitochondria.*

It is general opinion that all visible parts of a cell which might be designated intracellular bodies, such as nucleus, mitochondria, neutral red granules, vacuoles, fat globules, ingested particles, globules of fluid, centrosomes and chromosomes, exhibit passive and not active movements. The particles are carried by flowing protoplasm, or by local changes in viscosity of the cytoplasm. Rotation of the nucleus in late prophase and early metaphase is fairly common in fibroblasts in culture. Increase and decrease in viscosity, says Lewis (121), result in local changes in the contractile tension of the part of the cytoplasm involved. The cytoplasmic matrix ordinarily shows no visible signs of these local changes in viscosity, but it is assumed that they occur. Such changes result in local displacement of the intracellular bodies; conversely, the fact that the various intracellular bodies are displaced and move about in the cytoplasm is possibly an indication of invisible local changes in viscosity of the cytoplasm.

It is probable that passive movement of cell inclusions is accomplished by protoplasmic flow, or contractility without change in viscosity.

Nucleoli show but little movement in the resting cell. During mitosis they exhibit a slight motion as they split apart in prophase. This is again probably a purely passive movement.

In contrast to the foregoing is the view of Valkanov (199) who says that movement of nuclei and chromatophores is autonomous. He believes motility is accomplished by secretion of substances, as in some lower organisms.

I do not wish to imply that nuclei, chromatoplasts or other cell bodies possess independent motility as a normal function, but I do believe that the possibility of automaticity on the part of cell particles must be recognized, to support which I should like to record an observation. An echinoderm egg awaiting fertilization is, ordinarily, a wholly passive cell, yet such an egg, of *Tripneustes,* underwent amoeboid movement without apparent cause. It first put out

a blunt pseudopod, then slowly changed form, progressing the while, until after several minutes its contour was as irregular as that of an active amoeba.

If a cell such as an egg, for which amoeboid movement is seemingly a wholly functionless activity, can become motile, then how much more likely is it that a nucleus or chloroplast, for which motility serves an important function, may assume amoeboid or other motion when necessary. To accomplish diploidization (29) by passing through the pores of cross walls, the nuclei of certain fungi may become amoeboid. It is, of course, also possible that these nuclei are passively carried by streaming plasmodesmata.

D. *Chromosomes.*

Movement of chromosomes has long been a problem in cytology. Amoeboid movement, electrical forces, surface tension and contractility of spindle fibers are some of the forces resorted to in explanation. If contractility of spindle fibers is responsible, then the fibers must first pull the chromosomes into the metaphase plate, at which time oscillation of the chromosomes in and out of the metaphase plate is to be seen. Then during anaphase, the chromosomes must be pulled to the poles again by increase in the contractile tension of the spindle fibers. That spindle fibers exist and are attached to the chromosomes seems likely, but more than this cannot be said.

E. *Membrane.*

Streaming of the surface layer of a protoplast bears interestingly on the reality of the protoplasmic membrane. I (173) have repeatedly emphasized that the cell membrane is protoplasm and may undergo the same physical changes as does the inner protoplasm which it bounds. Both the outer surface layer of protoplasm and the inner vacuolar membrane or tonoplast are formed of protoplasm. These layers differ, to be sure, chemically and physically from the inner protoplasm because of forces active at surfaces, but they are, nevertheless, living protoplasm.

Cell surfaces are likely to be coated with a thin film of oil, but this is not to be confused with the more substantial morphological protoplasmic membrane, a structure that may, in certain instances, be lifted off. That the protoplasm of which the membrane is composed exhibits streaming, is a fact of great physiological significance

and helps toward an understanding of the true physical nature of the protoplasmic surface.

Biologists use the term cell membrane rather freely to mean the outer layer of a protoplast, meaning thereby not simply a surface tension film, but a morphological "skin," capable of being lifted off under favorable conditions.

With the introduction of new concepts, it is now very difficult to know just what each author has in mind when he says "cell membrane". Mast (128) postulates a "plasmalemma" which subtends a "plasmagel" enclosing a "plasmasol". Some use "plasma membrane" to indicate the surface layer which encloses a cell and gives to it the properties of selective permeability, absorption and excretion, but which is a structure that cannot be seen under the microscope; and they use "cell membrane" to indicate a structure which can be demonstrated histologically as a definitely stainable line. Scarth describes a surface film and a layer of cortical endoplasm (see also II-6-E).

When an amoeba advances, the entire surface layer flows, and it is precisely in amoebae that one of the best demonstrations of artificial lifting off of the cell membrane has been made. When amoebae and slime molds move forward, the advancing surface is in a constant state of change. As the surface increases, material is added to it from the inner protoplasm, and as the surface decreases, part of its substance is returned.

The following observation led one of my students to flatly deny the existence of the tonoplast, when it should have taught him to recognize that protoplasmic membranes are alive, dynamic and not static. This student had observed a particle within the vacuole of a plant cell moving at the same rate and in the same direction as the streaming protoplasm within the primordial utricle. Close observation revealed that the protoplasm which was in direct contact with the vacuolar sap was in an active state of flow, as was the inner protoplasm. It was the streaming interfacial protoplasm which caused movement of the vacuolar sap and the particles within it. There was for him, therefore, no quiet layer between protoplasm and sap, which meant to my student that there was no tonoplast. He failed to appreciate that the tonoplast and cell membranes, in general, are not inert skins but surface layers of active protoplasm. Schorr (159) supports this, for he observed crystals of anthocyanin

in the vacuoles of *Allium* cells and saw these crystals move, carried by the flowing protoplasm of the tonoplast.

The tonoplast is, as Hugo de Vries said, "a membrane differentiated and living".

F. *Cilia.*

Cilia are the only cell parts so far considered, the movement of which is not only above question, but is the chief characteristic and function. Their whipping motion is not a form of protoplasmic streaming, but it may involve streaming; at least, such an hypothesis has been advanced. The suggestion made is that the cilia are hollow and are activated by inward and outward flow of protoplasm. Aside from the question as to the reality of the tube-like nature of cilia, the flow of protoplasm into a flexible tube cannot cause continuous and rhythmic whipping of the cilia unless that flow occurs in exceedingly rapid pulsations, for which there is as yet no evidence. A rhythmic progressive and regressive flow of protoplasm occurs in some organisms (174), but the cycle is not comparable in time with the rapid movement of cilia.

III. TYPES OF STREAMING

1. Agitation.

Protoplasm often exhibits a feeble general activity that I have called agitation, for want of a better word. It resembles the movement of a fluid which is slightly disturbed. Agitation probably corresponds to that motion which in German has been called "Glitschbewegung". The movement is a churning one and often very active. I place agitation first on the list of types of streaming because it is both the most neglected and probably one of the most common forms of protoplasmic activity. De Vries (211) believed that as long as a cell could generate energy its protoplasm would be in active movement. Arthur (8) describes local disturbances in protoplasm.

Such local turbulent movements may be due to changes in osmotic pressure, metabolic activity, surface changes causing displacement within, or a variety of other causes.

Agitation appears to be an unorganized form of protoplasmic movement. Analysis of it is therefore extremely difficult. It is, however, significant because it may represent the most common

type of protoplasmic movement in that it is constantly present in all physiologically active cells.

2. Rotation.

In cells such as those in *Elodea* leaves where the protoplasm is confined to a peripheral layer, to a primordial utricle, the movement of the protoplasm follows the contour of the cell. This is rotational streaming. Under normal conditions, the flow is in one direction in the same cell, that is, clockwise or counter-clockwise. Rotation is a common type of protoplasmic flow, and one of the most orderly. It has been the subject of much study and the basis of most theories of protoplasmic flow.

3. Circulation.

In cells such as those of *Spirogyra* and of parenchymatous tissue, where the protoplasm is more generally distributed with a central mass enclosing the nucleus connected by strands to a peripheral layer, flow takes places in numerous directions at one and the same time. This is circulatory streaming. It is perhaps the most common form of organized protoplasmic flow in definite currents, but one of the least orderly. So haphazard is the direction of flow that circulatory streaming appears to be devoid of directional control.

The distinction between rotation and circulation is not a basic one, that is, the two do not present fundamentally different types of movement, but merely two patterns of flow, two expressions of protoplasmic distribution. Rotational streaming takes place because the protoplasm is in a single layer held against the wall by the central vacuole. There is no other path for the flowing protoplasm to follow than that of the inner surface of the wall. In cells exhibiting circulatory streaming, protoplasmic strands cross the vacuole, presenting numerous diverse and irregular paths which the moving protoplasm may take. Hauptfleisch (81) reports that circulatory streaming passes into rotation whenever the protoplasmic strands are made to retract so as to leave a single uninterrupted central vacuole.

4. Shuttle.

Shuttle streaming is common in certain lower organisms, notably in coenocytic forms, as the Myxomycetes and Phycomycetes. This type of streaming is often referred to as "one-way" flow. Both

expressions imply that movement is in one direction only at a time. Reversal in direction of flow must take place, but there is no return current at the time that the forward or backward flow is under way.

A pure case of shuttle streaming takes place in the slime mold, *Physarum*. It has been attributed to other coenocytic forms such as *Vaucheria* and *Rhizopus*, but what has been assumed to be a one-way flow in *Mucor* is most often actually a rotation, for there is a return current of one sort or another.

German scientists use the expression "flutende Strömung", or tidal flow, without always being clear as to what is meant, for with tidal flow there is sooner or later a return current. "Flut und Ebbe" would be a more accurate characterization. It is possible that some authors in referring to tidal streaming have had in mind an irregular forward surging of protoplasm, a rushing in one direction, followed, not necessarily by a return, but by a lull and then another rush forward. Such movement often occurs, indeed it is rather frequent, in the shuttle type of streaming. But there is no tidal flow of protoplasm without an ebb flow.

Arthur (8) describes the movement in *Rhizopus* as a surging precipitate one. Of the return current in *Rhizopus* Arthur says it is occasionally to be seen, is often well defined, and always occupies the periphery of the hyphal cavity. It carries no vacuoles, as these have probably been used in growth or extravasated. Between the two streams there is a quiescent layer of protoplasm of about the same thickness as that lining the walls.

5. Sleeve.

The sleeve type of protoplasmic flow is very interesting and has been referred to in the literature (162). It occurs in coenocytes. Movement is in one direction through the core of the hypha accompanied by a return flow in the cortical layer. The protoplasm lining the walls thus forms a moving mantle around the inner flowing protoplasm.

This type of flow may easily be mistaken for another which is intermediate between it and a rotational form of streaming known to occur in the hyphae of *Mucor*. Ordinarily, rotational streaming is characteristic of cells with a primordial utricle surrounding a large central vacuole, but it may take place in hyphae as well. Because of this, considerable confusion has arisen over the type of flow in *Rhizopus* and related genera by failure to observe the streaming

with sufficient care. At times the entire protoplasmic mass moves in one direction only. But often there is a return current, which may be one of several kinds; it may be in the cortical layer, *i.e.,* the sleeve type of flow, or the return current of protoplasm may fill half of the hypha, a semi-cylindrical section, the other half cylinder of protoplasm flowing in the opposite direction. The two currents touch at an imaginary stationary layer which divides the hypha into longitudinal halves.

Again, if the outer cortical layer in the sleeve type of flow does not wholly envelop the inner core, but only partially surrounds it like a cuff, then we have another arrangement of streaming protoplasmic layers which is intermediate between the rotational and the sleeve types of flow.

There are several forms of streaming which are readily induced but which may occur normally. Such are the spiral and belt types of flow. As my acquaintance with these is solely in treated material and as other experimenters have given no definite examples of them in normal material, I shall treat of them under Pathological Forms of Streaming (VI), without wishing thereby to deny their possible occurrence as a normal type of flow.

Though the mode of streaming exhibits divergent patterns, all types above mentioned are common insofar as they are concerned with the fact that protoplasm is subjected to a shear with the result that the interstructural relationships undergo continuous change.

In dermatoplasts, as are most plant cells, the outer contour of the protoplast cannot be changed as the protoplasm flows, whereas in gymnoplasts, as slime molds and some protozoa, streaming generally involves change in the form of the organism. The mechanics of flow in the two cases may be different, yet both are closely related phenomena, and the principles of the basic mechanisms may be the same. On this assumption, protoplasmic streaming in dermatoplasts and in gymnoplasts will be considered, in what follows, as parallel processes.

IV. DIRECTION AND RATE

1. Direction.

Every observer of protoplasmic streaming in *Elodea* has probably wondered if the direction of movement remains the same. According to Pfeffer (144), it does, and it is such as to be "in opposite directions on the two sides of the dividing wall between each pair of contiguous cells".

If the directions of flow in two adjoining cells are opposed, then it must be assumed that the direction in one is determined by the presence of the other. The position of cells in multicellular organisms determines in large measure their function and fate. This is a fully recognized concept, but Berthold (17) thinks that this principle does not necessarily apply to the direction of protoplasmic flow. He maintains that there is no constant relationship between direction of streaming in one cell and in another, but both Pfeffer (144) and Ewart (59) are convinced that there is.

In his work on the fungi, Buller (29) makes one very unexpected observation, namely, that the direction of flow of protoplasm in the mycelium of *Fimetaria* is in one direction only, from older hyphae toward rapidly growing younger hyphae, without any reversal.

Direction of streaming is sometimes determined by the shapes of cells and their orientation. Streaming normally takes place parallel to the long axis.

Ewart (59) adds that direction of flow may be reversed by injury or by the death of neighboring cells.

2. Rate.

The rate of protoplasmic streaming in the slime mold *Physarum polycephalum* I have found to vary usually between 0.3 and 0.6 mm. per sec., when measured midway between reversals, *i.e.,* at maximum flow. The highest value given it by Kamiya (101) is 1 mm. per sec. Vouk (210) records 1.25 mm. per sec. as the maximum rate of flow in slime molds. This appears to be the fastest known rate of protoplasmic streaming. Much slower rates are reported by Hofmeister (87) who names 5.4 mm. per min. for *Physarum.* He also gives 1.5 mm. per min. for *Vallisneria* and *Elodea.* Ewart reports 2–3 mm. per min. for *Chara* and *Nitella.* The rate of protoplasmic flow in *Rhizopus nigricans,* as given by Arthur (8), is 3.3 mm. per min. which is twice as fast as in *Nitella* and four times the speed in *Tradescantia,* but less than that in Myxomycetes.

Bushee (33) made a large number of measurements of streaming rates, most of which are surprisingly low. He does not average the many readings, but a glance indicates an average of about 0.2–0.3 mm. per min. or 0.003–0.005 mm. per sec. The maximum rate of streaming at 20° C. occurred in *Nitella* with 1.6 mm. per min. (0.027 mm. per sec.); the "optimum" at 34° C. was 2.84 mm. per min. (0.047 mm. per sec.).

The discrepancies in rate values given by various authors are due to one of several errors. There is, first, the failure to realize that rate of streaming varies greatly and is dependent upon temperature, gas exchange, respiration, hydration, chemotaxis, light and innumerable unknown internal reactions. Also, in the normal, undisturbed, uniform streaming of protoplasm in the shuttle or one-way type of flow, the protoplasm must come to a full stop at the end of each rhythmic period in order to reverse its direction of movement. There is, consequently, a continuous change in rate of flow from zero to maximum speed to zero again. Still another error which frequently creeps into measurements of streaming rate is failure to measure the average velocity of the fastest particles, rather than the average velocity of all particles. In general, only the movement of small particles which are not deterred by friction give an accurate indication of the rate of flow of a mass of fluid. One would not measure the streaming velocity of a river by the movement of any one log chosen at random when some are deterred or stopped by friction, but rather by the movement of free logs only.

It is customary to distinguish between rate or velocity of protoplasmic streaming (48) and intensity, *i.e.*, the amount of protoplasm in motion (22, 200).

Kinoshita (103) terms the quotient of the equation, time of progressive streaming/time of retrogressive streaming, the "streaming rate" of a slime mold. The quotient is a measure of the rate of advance of a plasmodium.

A tabulated summary of measurements of rate of protoplasmic flow is given below. The readings are from Vouk (209), Bushee (33) and Pfeiffer (145).

ORGANISM	STREAMING RATE IN MM/SEC.	EXPERIMENTER
Didymium	1.250	Vouk
Physarum	0.090	Hofmeister
Mucor	0.055	Arthur
Nitella	0.050	Ewart
Nitella	0.027	Naegeli
Vallisneria	0.026	von Mohl
Elodea	0.015	Ewart
Tradescantia	0.013	Hofmeister
Elodea canadensis	0.010	Bushee
Cucurbita	0.008	Hofmeister
Tradescantia zebrina	0.006	Bushee
Urtica	0.005	Hofmeister
Avena sativa	0.005	Bushee
Sagittaria	0.004	von Mohl
Vinca major	0.002	Bushee
Potamogeton	0.0001	Hofmeister

	RATE OF LOCOMOTION IN MM/SEC.	
Paramaecium	1.3	Pfeiffer
Euglena	0.1	Pfeiffer
Spermatoboan	0.03	Fisch
Bacillus	0.01	Pfeiffer
Amoeba	0.0005	Pfeiffer

The rate as well as the direction of flow in slime mold can be accurately controlled by establishing a pressure difference (see VIII, 3). By this method Kamiya (100, 101) raised the velocity of protoplasmic flow to more than 2 mm. per sec., which is, though artificially accelerated, the highest velocity of protoplasmic streaming yet known.

V. KINOPLASM *vs.* MATRIX

The idea that only part of protoplasm flows, that only part is the active, streaming, living substance, and the rest a matrix or solution bathing the dynamic component, has long prevailed in biology. Strasburger differentiated between two such parts and called the active portion "kinoplasm" and the passive medium "spongioplasm". Scarth (158) has of late supported this view so well that it will be sufficient to refer to his writings alone.

In a diagrammatic cross section of the cytoplasm of a plant cell, Scarth (158) differentiates: *a*) the cell wall of cellulose; *b*) the ectoplast or protoplasmic surface film (this is probably what heretofore has been called the plasma membrane); *c*) plasmagel layer or cortical endoplasm; *d*) plasmasol or liquid endoplasm (this is the above referred to matrix and apparently identical with what Strasburger called spongioplasm); *e*) the kinoplasm, threading its way through the matrix; *f*) the tonoplast or vacuolar membrane; and *g*) myelin processes protruding into the vacuole as part of the tonoplast.

There is no attempt on the part of Scarth in drawing a distinction between active and passive plasm to imply that the protoplasmic granules are automotive, a view held by some (199). He is convinced that the moving granules lie in and are carried by streams of protoplasm. The question is, how much of the protoplasm is involved in movement? Scarth answers this with two statements: it varies, and is rarely all of the protoplasm. Careful observation with a narrow aperture reveals streams of a substance which has a refractive index different from that of the surrounding protoplasm;

the former is kinoplasm, the latter matrix. Streaming, says Scarth, is confined to kinoplasm. The matrix is stationary unless activity of the kinoplasm induces a passive flow of it.

The kinoplasm joins and at times appears to arise from the surface film. The surface film itself may slip along independently of the nonmotile endoplasmic matrix. The intimate connection between kinoplasmic threads and the substance of the surface film, and the tendency of cytoplasmic surface films to form myelin processes, leads Scarth (157) to the belief that there is some similarity in the composition, molecular structure and activity of surface films and inner kinoplasmic fibrils.

In this connection it is worthy of note that Booy (20) also views the protoplasmic membrane as a complex system involving far more than a surface tension film.

Whether or not an active kinoplasm and a passive matrix will prove the rule in cells, generally, remains to be seen. Certainly in some cases, as in fungal hyphae, there appears to be a mass movement of all the protoplasm (29), but Scarth believes that this is usually not the case.

VI. PATHOLOGICAL FORMS OF STREAMING

Cells are in an abnormal environment whenever excessive changes in their environment occur. If reagents are applied, temperature or light changed beyond the normal range, the streaming of protoplasm is to be regarded as pathological. The effects of injury are likewise pathological. But cells may be abnormal from inner causes, such as faulty metabolism. These are the subject of the present section; the effects of artificially produced changes in environment are dealt with in subsequent sections.

Before turning to inner causes of pathological types of protoplasmic flow, I should like to dispel one idea which persisted in physiology about three decades ago, namely, that protoplasmic flow, in general, is pathological.

Those who held the pathological view stated that as movement is brought on by externally applied reagents, and by injury, such as cutting or the severing of a leaf from the stem, the flow was obviously pathological. This is bad experimental work and unsound reasoning. Streaming may be seen in the undetached leaf. Wounding or any form of shock may arouse a cell into activity, but activity at other normal times is not so aroused. If a plant is

aroused into activity during the winter's rest, this is no indication that growth in summer is pathological.

The extraordinary belief that protoplasmic streaming is pathological prevailed for some time. Why, it is difficult to understand, when such able workers as de Vries had earlier held that protoplasmic flow was of common occurrence and was exhibited by all cells at one time or another in their lives. De Vries actually went further and maintained that only when a living cell was too feeble to generate the necessary energy was its protoplasm ever at rest. Velten (200), in 1872, pointed out that protoplasmic streaming is a normal process and widely distributed in the plant kingdom, in tissue cells, in hair, in leaf epidermal cells, slime molds, pollen tubes, fern prothallia, algae, *etc*. If to these observations and opinions we add the fact that in forms such as *Amoeba* and Myxomycetes streaming is a striking and constant characteristic, then it becomes absurd to regard protoplasmic streaming as pathological. This statement in no way contradicts the existence of pathological forms of protoplasmic flow, which is our interest at the moment.

One is often put to it to decide when an extreme condition is pathological and when not; thus, cessation of protoplasmic streaming from cold when induced in the laboratory may be pathological, but when the same occurs out of doors, it cannot be regarded as pathological if frequent and without damage. When certain naturally occurring environmental factors produce changes in type and prevalence of protoplasmic flow, these are abnormal only in so far as they are unusual.

Some very striking forms of protoplasmic streaming may be induced through application of reagents. Two of these which are frequently to be seen in treated cells are the spiral and belt type of flow.

The spiral type of streaming is so unusual that I at first was inclined to regard it as wholly pathological, but it may possibly occur in normal cells. It was first cited by Amici (3) who thought it due to a twist in the cell walls, but as it can be induced in cells which normally show simple rotational flow, it is obviously not due to spirality of the wall. Braun (24) attributed spiral streaming in *Chara* to torsion in the internodes. I believe this incorrect. Spirality is a deeply laid property of living matter. Spiral stream-

ing is more likely a characteristic of protoplasm than induced by the cell wall. Of course, like a bullet in a rifle, it would be given a spiral twist by markings on the inner surface of the wall, but protoplasm shows spirality when no such markings are there. Spiral protoplasmic streaming occurs often in material treated in salts.

A second type of unusual protoplasmic flow is that of a belt around the "waist" of a cell. I know of it only in abnormal material (170). The belt type of flow is well illustrated in treated *Elodea* leaf cells. The chloroplasts seem to revolve like a barrel in water.

These two unusual types of protoplasmic flow are often seen in plasmolysed (Küster, 112) and in alcohol and strontium chloride treated plant tissues.

Injury may influence streaming, but much less so than anticipated. A microneedle inserted in a plasmodium and moved about kills the protoplasm with which it comes into immediate contact, yet leaves unaffected the streaming protoplasm just without the area traversed by the needle.

Schröter (162) and Andrews (7) report that injury does not produce or accelerate streaming, but has a tendency to decrease it.

Hosoi (90) found that cutting narcotized paramecia at various points caused no interruption of protoplasmic streaming on recovery of the fragments. Continuation of streaming in isolated bits of protoplasm has been noted by a number of investigators. Jost (94) reports this in reference to droplets of *Chara* protoplasm. If *Amoeba* is sectioned, streaming recommences in the fragments, whether or not the nucleus is present.

The effect of injury on protoplasmic streaming will obviously depend on the sensitivity of the organism and the extent of the injury. Furthermore, it is not true that wounding always causes the same effect on protoplasm, as apparently assumed by Peterfi and Yamaha (143) who regard liquefaction, followed by thixotropic setting, as the expected result when protoplasm is mechanically disturbed by a penetrating needle. They thought the irreversible coagulation which took place on the microdissection of *Nitella* cells as a special case. This is by no means true; coagulation is as likely to result from mechanical injury as is liquefaction in any protoplast. Streaming will be affected in either case.

VII. INFLUENCE OF INTERNAL FACTORS

1. Cell form.

The shape of a cell may determine the extent, the rate, and the type of protoplasmic flow. Thus, if a cell is spirally formed or marked, the protoplasm within it is likely to flow in a spiral path.

Though spiral flow is not necessarily dependent upon spiral wall markings, yet were these present they would influence streaming; at least one would be very much inclined to assume that the two are correlated. With this, however, Kirchheimer (76) disagrees. He says that the sporangia of *Phycomyces* show a right-hand twist whereas the protoplasm flows counterwise. But Oort and Roelofsen (141) believe spiral growth and streaming are related.

Streaming is not frequent in young cells, though in some, as cambium, it is quite active. Where absent, it is possible that the great internal resistance to flow in small cells is responsible. Streaming is usually most active in highly vacuolate cells and meristematic tissue is relatively free of vacuoles.

2. Viscosity.

It is obvious that the rate at which a liquid flows is determined in part by the force back of it and in part by its viscosity. If one of these factors changes, the rate of flow changes. Students of protoplasmic streaming have often neglected the effect of the motive force, being too ready to assume that a change in rate of protoplasmic flow is due solely to a change in viscosity. In all probability a change in motive force is more often responsible for a change in rate than is viscosity. When the protoplasm of a slime mold reverses its direction of flow it must slow down to zero and then speed up again to maximum rate. As protoplasm is non-Newtonian in its behavior (173) there will be a change in viscosity, but that change is the result and not the cause of the change in speed. Viscosity changes if the motive force changes, because of the non-Newtonian nature of protoplasm, and rate of flow will change if the motive force changes, but far less often does rate of flow change because of change in viscosity. However, the effect of viscosity on protoplasmic streaming is important, even though viscosity is too often assumed to be the cause of change in rate.

Extensive studies on the relationship between viscosity and rate of protoplasmic flow were made by Ewart (59), Bělehrádek (15),

Lambers (115, 116) and Romijin (154). Both Lambers and Romijin emphasize viscosity as the factor responsible for change in streaming rate. Lambers states that increased rate of streaming at high temperatures is due to progressive decrease in viscosity, and Romijin regards the greater viscosity which he believes to have observed at 20° C. as accounting for the transitory slowing down of streaming. Any one of a number of physiological factors may have been responsible rather than a change in viscosity, which can be established in *Nitella* with great difficulty. Aside from this objection, it is extraordinary to find maximum viscosity and minimum velocity at 20° C., with lower viscosity and greater velocities at both higher and lower temperatures. The optimum temperature for growth is close to 20° C. Cold almost invariably increases protoplasmic consistency and slows down streaming, but whether streaming decreases because viscosity increases is another matter. Jungers (97) noted lowered viscosity due to injury in *Allium*, but Schorr (159) observed slower streaming under the same conditions; consequently, if both are correct, lowered viscosity does not mean faster streaming.

3. Cell organization.

In discussing types of streaming, it was said that protoplasmic movement in young cells is often of the circulatory type without any defined direction, but that as the cell matures and acquires a large central vacuole, the type of streaming becomes rotational, assuming a well defined course. Thus does the inner organization of a cell, the arrangement of its parts, determine the character of streaming. The reverse situation is presented by Cazalas (37) who states that the evolution of the vacuome in *Chara* and *Nitella* is determined by cytoplasmic movements.

4. Transverse walls.

In Chapter II of his "Researches on Fungi", Buller (29) gives a review of the translocation of protoplasm through the septate mycelia of certain Pyrenomycetes and other fungi, wherein he discusses at length the streaming of protoplasm from cell to cell by means of plasmodesmata.

Protoplasmic connections from cell to cell have been the subject of much discussion, their existence being severely questioned, until finally even those who most strongly opposed them now agree that

living protoplasmic threads do pass from cell to cell. Streaming in these threads was discovered in fungi in 1900 by Ternetz (191) who observed unhindered flow from cell to cell, and from one system of hyphae to another *via* anastomoses under favorable conditions, passing through twenty or more hyphae. From this and his own work, Buller concludes that "all living cells which make up an individual plant are connected together so as to form a single mass protoplasm", and from this fact Buller says it is possible to understand how a multicellular plant can develop and react to external stimuli in a unitary manner.

An interesting feature of the streaming of protoplasm through cross walls is the manner of passage of vacuoles of good size through pores in the septa. They do so by squeezing through and thus becoming greatly constricted, or by budding off small bits as they pass through.

Still another important feature of protoplasmic flow through cross walls is speed. Buller (29) states that the rate of flow of protoplasm in septate hyphae is the same as that in non-septate hyphae. The protoplasm must, therefore, pour through septal pores with great ease.

5. Age.

The activity of most vital organs is affected by age. The extent to which protoplasmic flow is influenced by age is unknown. There have been suggestions, but these indicate increased rather than the expected lessened activity. Such an interpretation may be due to an error which might arise from the assumption that active streaming in older cells as compared with feeble streaming or lack of it in very young cells means increased activity. But, as we have seen, this is true only in one sense. Young cells show little protoplasmic movement because of higher internal resistance of small cavities and because of absence of vacuoles; thus, it is not primarily age as such but size and internal organization which is responsible for a higher rate of flow in old cells. Vesque-Püttlingen (206) also finds that rate of protoplasmic streaming in the root hairs of Hydrocharitaceae gradually increases with age; and Vouk (209) states that the rhythmic period of streaming increases with age in slime molds.

There are changes in protoplasmic flow which are associated with

age; thus, with approach of fruiting in slime molds there is decrease in streaming rate, but if there is to be no fruiting, there will be no change in rate with age.

VIII. EFFECTS OF EXTERNAL AGENTS

1. Temperature.

It is generally agreed, as Lambers (115) noted, that the rate of streaming, *e.g.*, in *Nitella*, is accelerated by higher temperatures within a definite range.

Clifford (43) found that the protoplasm of slime molds survives between temperatures of – 2° and 52° C., and is positively thermotropic up to 33° C., but negatively so above this point.

These temperatures must represent extremes, for at – 2° C. streaming ceases and at 52° C. slime mold protoplasm dies; indeed, plasmodia succumb within a day or two at temperatures above 35° C.

Cook (47) found a rapid change in rate between 20° and 35° C. At 10° C. streaming stops quickly and recommences in two minutes. The author concludes that a definite fall in temperature of 15° to 20° is necessary to stop flow, that recovery follows a definite course dependent upon temperature, and that it is not cold as such but temperature shock which brings about cessation of streaming.

Hill (85) pointed out that when temperature is suddenly lowered from 36° to 20° C. in *Nitella* the action current is raised, the protoplasm gelatinized, and streaming stops.

Bushee (33) recorded streaming rate at two temperatures, 20° C., and the temperature of optimum flow (30° to 38° C.). In every case the greater rate was at temperatures above 20° C., thus showing that streaming is accelerated by heat. In no case did he fail to get a greater rate at higher temperatures.

Schröter (162) found that a rise in temperature hastens streaming in mucors or may initiate it. Cooling may cause streaming to cease. A high temperature results in backward streaming (this is an extraordinary and little reported behavior). At about 55° C. streaming ceases and the mycelium dies.

In slime molds (from my own unpublished work) injury sets in at 35° C., streaming stops at 40° C., and death occurs before 45° C. is reached.

Optimum and minimum temperatures for streaming in *Mucor* are

26° C. and 10° C., says Schröter, but I find that streaming in slime molds continues down to 2° C.

Bottelier (22) found that the rate-temperature curve is logarithmic between 7° and 25° C. with a Q_{10} of 1.8 in a 140–200 hours' old coleoptile. Above 25° C. the Q_{10} is smaller. Coleoptiles 90 hours old gave a logarithmic curve up to 17° C., but from 17° to 35° C. the rate remained constant.

The amount of research on the effect of temperature on streaming is great and includes, in addition to the foregoing, work by Beikirch (14), Romijin (154), Olson and Du Buy (140) and especially Velten (203).

Gehenio and Luyet (73) report cessation of streaming in slime molds at 5° to 0° C., followed by protoplasmic disorganization below freezing.

The great variability in the foregoing work is due in part to the different kinds of material used, in part to failure to distinguish between temperature as such and temperature shock, in part to the condition of the material, and undoubtedly to other neglected and unknown factors.

That temperature shock due to the suddenness rather than to the amount of change is responsible, is indicated by Andrews (7); he says that a sudden change in temperature of several degrees will cause streaming in fungi.

That there is full coincidence between van't Hoff's law and streaming has been shown by a number of workers. Vouk (210) showed this for the rhythmic frequency of streaming; he found a Q_{10} value of 2–3. Cook (47) determined the temperature coefficient of recovery and obtained a Q_{10} value of 1.5.

2. Visible light.

Light affects protoplasmic streaming, but much less so than anticipated. If the light is intense, streaming slows down, probably not due directly to the light, however, but to secondary effects.

Fitting (63, 65, 66) termed the stimulating effect of light on protoplasmic streaming, "photodinesis". Pringsheim (150) showed that intense light retards and eventually stops streaming in *Nitella, Tradescantia* and *Spirogyra*. Ewart (59) made similar observations on *Chara, Elodea* and *Vallisneria*. Schweickerdt (159) demonstrated that light may initiate streaming, as little as 0.5 meter

candle having an effect. Red light was the most effective, then blue and green. Infra-red rays were without effect. Bottelier (21) found blue light to have the greatest effect on rate of streaming in the coleoptiles of *Avena,* then violet, ultraviolet and green; yellow light caused no perceptible effect.

Nothmann-Zuckerkandl (139) finds that intense light arouses streaming in *Elodea.* Visible rays, ultraviolet and infrared all function as activators of streaming. Quantitative measurements indicate that the power of activation is a function of the wave length. That longer rays are more effective suggests that heat is the responsible factor, but Nothmann-Zuckerkandl finds that applied heat does not always arouse cells to active streaming.

Schröter (162) found that, in general, light has little influence on the movement of protoplasm in mucors other than sudden exposure to light from darkness, which may initiate or hasten streaming. My own interpretation of sudden exposure to light would be to regard the result not as a light effect but as shock. Any shock, whether by light, heat or electricity or mechanical, would probably accomplish the same result.

It is possible that tissues commonly exposed to light, such as mucors and Myxomycetes where, in the latter, I find no stimulating effect on streaming by light, are not activated by light, whereas tissues not naturally exposed to light, such as the inner cells of stems, are sensitive to light and respond by an increase in streaming.

Andrews (7) reported that light may initiate and accelerate streaming when alternated with darkness in fungal filaments in a condition for streaming. Beikirch (14) maintained that the stimulating effect of light on streaming manifests itself by initiating streaming in quiet cells and not by increasing rate of flow. The number of cells aroused and not speed is the effect to be noted. Intense light decreases rate of flow.

3. Ultraviolet light.

Investigators of the effect of ultraviolet light on protoplasm include Schulze (166), Vouk (210), Gibbs (75) and Noethling and Rochlin (136). Gibbs (75) found that radiation of 312.6–237.8 mμ first causes liquefaction and then coagulation of the protoplasm of *Spirogyra.* Vouk (210) finds ultraviolet light very damaging, one minute being sufficient to stop flow in plasmodia. Five minutes is sufficient to kill.

Work on ultraviolet is of little value if the bands or wave lengths are not given, for the effect of ultraviolet light varies greatly with the parts of the spectrum used.

4. Water.

The effect of water on streaming should be negligible except in so far as its presence in the inner and outer environment is necessary for streaming to continue. There has crept into the literature the erroneous opinion that distilled water in itself stops protoplasmic flow, and that cessation of movement under these conditions is due to excessive end- or exosmosis of water. This cannot be true because water applied gently to protoplasm so as to cover it fully but slowly does not stop flow. The cessation of movement occurring at immersion is due to shock (see Section VIII–14) by the pressure necessary to break through the surface tension of the water film. In 1878 Strasburger (185) discovered that a plasmodium will move against a current of water. Five years after Jönsson (93) corroborated this and named the reaction "positive rheotropism". In the following year Stahle (184) gave an unsatisfactory explanation. A decade later Clifford (43) repeated the work and measured the force against which protoplasm will flow.

A botanist, recalling hydrotropism of roots, might assume that positive rheotaxis is simply positive hydrotropism. That this is not true is indicated by pure reasoning and by experimental work. Were positive rheotactic response on the part of a plasmodium simply a case of seeking water, this would be as well accomplished and with less expenditure of energy by going with the stream rather than against it. Keferstein (102) has experimentally shown that a plasmodium creeps upward against a stream of water even when in a water-saturated environment. She emphasizes that rheotaxis is not to be confused with hydrotaxis. Plasmodia are positive in both respects and show rheotactic response in a water-saturated atmosphere.

5. Salts.

Fitting (63, 66) termed the stimulation of chemical substances to protoplasmic streaming "chemodinesis". Much work on protoplasmic streaming, including that on the effects of salts, comes, then, under chemodinesis.

Among the surprisingly little amount of work done on the effects of salts on protoplasmic streaming is that of Seifriz and Uraguchi (181), Colla (45), and some of my own (170). The work of Seifriz and Uraguchi dealt primarily with toxicity of heavy metals, the time required to stop protoplasmic flow being used as an indicator of toxicity, and obtained the following toxic order:

$$Ag > Hg > Cd > Tl > Cu > Pb > Zn > Y > Sr > La > Rb$$

Elodea leaves when soaked in 1%–2% barium or strontium chloride for several hours or longer, depending on concentration, are aroused to pronounced and often abnormal activity (170). Barium is more effective and more toxic. The abnormality is seen principally in the rate and type of flow, and in the distribution of streaming. Certain parts of the cell protoplasm remain quiet while adjoining parts are actively streaming. The unusual types of flow are of several kinds, the most startling being a centrally located belt of streaming protoplasm and a spiral path taken at a very high rate.

6. Acid and alkali.

Colla (44) studied the effect of acidity on protoplasmic streaming. She found that the maximum rate of flow in the internodal cells of *Chara* occurs at pH 5.3 and the minimum pH 6.2. My Italian may be at fault, but a minimum flow at pH 6.2, which is the optimum for growth of many aquatic plants and certainly not damaging to most, would hardly seem to be the pH at which flow is at its lowest rate in a cell noted for its protoplasmic activity.

Robbins (153) found minimum flow in *Elodea* at pH 5.6. Pfeiffer (145) calls attention to this unexpected finding, noting that if there is, as Robbins maintains, a direct dependence of streaming on viscosity, then minimum flow should occur at the isoelectric point which is the point of maximum viscosity, and pH 5.6 is hardly the expected isoelectric point of protoplasm.

Strugger (188, 189) also found minimum flow in *Hordeum* rootlets at pH 6.15 and 5.65, thus supporting both Colla and Robbins.

These surprising results and the divergence among them may be due in part to the buffers used for controlling pH, the salt effects having been neglected.

Becker (13) compared the effects of various acids on protoplasmic streaming in the staminal hairs of *Tradescantia*. He found that streaming is stopped at different pH values, according to the acids used.

Coman (46) studied the effects of acid and alkali on slime mold protoplasm from the point of view of chemotaxis in order to supplement his studies on leucocytes. Emoto (57) had stated that varying chemotactic responses resulted with variations in concentration of H and OH ions. Coman (46) found that any chemotactic response obtained was always in the same direction. Both Emoto and Jochims (58) reported positive chemotaxis to weakly acid solutions and negative chemotaxis to strongly acid solutions. Their results, therefore, depend upon concentration of the acid. Coman found negative chemotaxis to both weak and strong acid solutions. His results seemed, therefore, to depend upon the H and OH ion, regardless of concentration, so long as it was adequate to cause any response.

Coman also found that a small bit of the slime mold comparable to a macrophage could be made to alter its response from positive to negative. When doing so the organism did not turn around, but "went into reverse". Although it cannot be stated that the chemotactic responses of Myxomycetes and leucocytes are identical, their mode of locomotion and chemotactic behavior are so similar that information gained from Myxomycetes may be applied to leucocytes.

7. Oxygen.

Much work has been done on the effect of oxygen on protoplasmic streaming. That this should be so is evident from the recognized fact that respiration is the source of energy for streaming (Section IX–2). If the amount of energy liberated can be accelerated or retarded by supplying more or less oxygen, then the effect of this would possibly be observable in the rate of protoplasmic streaming. Though there is much positive data, such as that of Clark (41, 42), Schuster (167) and Strugger (189), my own observations indicate that whereas some cells are sensitive to lowered oxygen pressure, others are not. The amount of oxygen needed by slime molds is so slight that in order to get an effect from oxygen the pressure must be greatly reduced, far beyond that usually possible in ordinary laboratory conditions.

Clark (42) found it possible to stop streaming in any healthy vegetative cell by depriving it of oxygen, and to restore streaming by a pressure of only 1 mm. to 3 mm. of oxygen, whereas I (177) have kept small plasmodia of *Physarum* in an atmosphere of what

I thought to be pure nitrogen for several hours without noticeable harm.

The explanation is given by Kitching and Pirenne (107) who found that the amount of oxygen necessary to keep streaming going in a slime mold is exceedingly slight. *Physarum polycephalum* stops in from 2 to 17 minutes after transfer to pure hydrogen. If the plasmodium is returned to air soon after stoppage of streaming, recovery is immediate, but if it is kept in pure hydrogen for a little while longer there is no recovery, the pigment diffuses out of the plasmodium, and death follows. Streaming is slowed down or stopped in oxygen-hydrogen mixtures of an oxygen tension of 3 mm. Hg, which is an oxygen tension such as might well occur in commercial nitrogen.

The poisonous effect of some toxic gases has been attributed to low oxygen pressure. This can be rarely true, except in cases of pure asphyxiation, and this is certainly not the cause of the toxicity of well-known anesthetics such as carbon dioxide. Oxygen may be added up to 90% and the toxicity remains.

An accelerating effect due to excessive oxygen is, in my experience, so slight that it cannot be observed with sufficient certainty to warrant a positive statement. And this is rather as it should be, for the living machine is adjusted to work at a given oxygen pressure. Any decrease in that amount will lower efficiency. An increase can have no effect, because maximum efficiency has already been attained and more oxygen cannot be handled. The atmosphere near the earth has always an oxygen supply which is in excess of that needed by protoplasm; to add more is without effect.

8. Organic substances.

Organic substances constitute a particularly attractive group of agents to apply to protoplasm. Many, such as the amino acids, are constituents of protoplasm, and many, such as the alkaloids, are poisons of great potency. The appeal has been so great that experimenters have tried most everything from quinine to snake venoms. The value of such investigations is not always immediately obvious, but in certain instances the results will undoubtedly have a practical medical or biological application. The stimulating or depressing effect of a chemical may be more quickly ascertained and more delicately determined by observing the reaction of streaming proto-

plasm to it than by noting the behavior of a higher organism as a whole.

Among the earlier workers to study the effects of organic substances on protoplasm was Planthaber (146).

The streaming of protoplasm in *Elodea* and *Nitella* was found by Marcy to be stimulated by concentrations of 0.25 to 0.075 gm. of ethylene chlorhydrin per liter.

Balbach (9) observed the effects of urea on the protoplasm of the slime mold, *Chondrioderma difforme*. He noted aggregation, changes in rate of flow, and swelling or shrinkage of the strands.

The work of Lepow (119) included a great variety of substances. He termed the study of the effects of alkaloids on protoplasm "protoplasmic pharmacodynamics". Lepow used the alkaloids cocaine hydrochloride, quinine hydrochloride, atropine sulphate, morphine sulphate, ephedrine hydrochloride and strychnine sulphate; the venoms of *Bothrops atrox* and *Crotalus atrox;* and chloral hydrate and epinephrine hydrochloride. He found that the initial effect of most of these reagents was temporary stimulation of streaming. Subsequent reactions varied. Quinine hydrochloride (conc. 1–150) caused complete cessation of motion a few seconds after the intial stimulation. Morphine (conc. 1–20) eliminated all motion in two to three minutes.

Fitting's (62, 63, 66) work in chemodinesis, as he called it, had to do with a variety of organic substances. He (70, 71) found that 0.00001 M sodium β-indoleacetate and 0.0000002 M methyl histidine induce streaming in *Vallisneria*. Various alpha amino acids (64, 67, 69), including α-asparagin (65), were tried, but they produced no effect at a dilution of 1 : 650,000,000.

Plant extracts prepared from the leaves of *Vallisneria,* Fitting (62, 68, 69) found to be effective in initiating streaming in dilutions of 1 : 2,000,000. He (70) believed their chemodinetic effect to be due to the presence of histidine.

Auxin is certain to be among organic substances applied to plants nowadays. Thimann and Sweeney (194) and Thimann and Schneider (193) studied the effect of auxin on protoplasmic streaming and found it to increase the rate of flow in *Avena* coleoptiles. Indole-3-acetic acid is more effective than potassium indole acetate. It brings out maximum increase in rate at 0.01 mg. per liter.

Other organic substances, such as ethylene chlorhydrin, histidine

and urea, in all concentrations, were without effect on streaming in *Avena* coleoptiles. No substance known to affect streaming had an effect comparable to that of auxin.

As the effect of auxin on normal streaming takes place at concentrations in the range of those which produce growth, it is assumed that the effect is closely connected with the effect of auxin on growth.

The accelerating effect of 2–4 dinitrophenol on streaming and respiration is in doubt. Olson and DuBuy (140) report that a solution of 50 mg. 2–4 dinitrophenol per liter causes a decrease of 44% in streaming rate in the oat coleoptile, or from 16 μ per sec. to 7 μ per sec. The same concentration decreases the respiration rate from 4.5 micro-liter per hour to 1.76 micro-liter per hour, or about 40% of the original rate. Concentrations of 0.01 mg. per liter and 1 mg. per liter of methylene blue, DuBuy (51) found to have no effect upon the streaming rate. Similarly, the same concentrations do not have any effect upon the respiratory rate, which DuBuy believes shows the close interrelationship between streaming and respiration.

Macht and Lubin (122) find that protoplasmic streaming in *Chara, Nitella, Tradescantia* and *Elodea* is soon stopped by treatment with menotoxin or menstrual toxin.

Watanabe *et al.* (214, 215, 217) found that the growth substances heteroauxin, *a*-naphthalin acetic acid, methylen blue, neutral red and glucose increase the potential difference between a plasmodium and the outer medium. Similar effects are obtained with *a*-methylheteroauxin, l-histidin, dinitrophenol and urea. The effect is supposed to be due to activation of oxidation-reduction metabolic processes. If, on the other hand, respiration depressants, such as hydrocyanic acid and copper sulphate, are applied, the potential difference is lowered.

9. Anesthetic agents.

Among the substances applied to streaming protoplasm few have yielded more interesting and instructive results than liquids and gases which produce anesthesia. Among the workers in this field have been Samassa (156), Nothmann-Zuckerhandl (138) and Vouk (210). Vouk found that a small quantity of ether accelerates protoplasmic flow in slime molds but a large quantity inhibits movement.

I (177) made an extensive study of the effects of ether, chloroform, acetone, carbon dioxide, cyclopropane, nitrous oxide, ethylene and other gases on the slime mold *Physarum polycephalum,* using cessation of protoplasmic streaming as a criterion of what I chose to call anesthesia.

"Suspended animation", or cessation of movement, when taken alone, is not the generally accepted indication of anesthesia, though it is the first definition of it. The usual criterion of anesthesia is loss of irritability; but it is very difficult to establish this in slime mold protoplasm other than through loss of movement. That loss of motility through application of certain chemical agents also means suppression of irritability, is hardly to be questioned when suspended animation is brought on by so well recognized an anesthetic agent as chloroform.

If, then, by anesthesia in slime molds is understood a rapid cessation of flow and quiescence for several minutes, followed by full recovery to active and normal flow without visible injury, then carbon dioxide, cyclopropane and chloroform are the ideal anesthetic agents for slime mold protoplasm. Thus, thoroughly anesthetized the protoplasm sets suddenly to a firm jelly. This was interpreted as a thixotropic gelatinization (72). That thixotropic setting to a firm jelly had taken place was shown by applying air pressure to one part of the plasmodium. Pressure readily moves normal protoplasm, whereas quiet carbon dioxide anesthetized protoplasm cannot be so moved.

On the basis of these results I (177) made the general assumption that anesthesia is due to reversible gelatinization of protoplasm. Gelatinized protoplasm is inert protoplasm. In such a state, most physiological activities, including movement, irritability and oxidative enzymatic reactions, are probably held down to a minimum. Furthermore, the chemical diversity of anesthetic agents makes it unlikely that anesthesia is due to a chemical reaction between drug and protoplasm but rather to a physical change.

10. X-rays.

Williams (219) finds that short exposure to Roentgen rays causes increase in the rate of protoplasmic streaming, whereas longer exposure results in decrease and finally stoppage. He (220) also finds that radium has similar effect.

11. Radium.

I (172) found that slime molds approach radium needles (dosages up to 12,000 r) freely when at a distance of several centimeters. The protoplasm advances more cautiously when within one centimeter; then, at about 5 mm. the advancing plasmodium stops and forms a front parallel to the needle. Here it remains for a time, perhaps several hours, then advances and flows over the needles (of 12 milligrams radium each), then retreats, and again advances to and over the needles, and again retreats, finally maintaining a cautious distance of 1 to 2 cm. Twenty hours of treatment from nine 12-milligram needles scattered in a 4-inch Petri dish culture is the maximum time of tolerance. When radiation is concentrated directly on a small plasmodium, all spreading stops, streaming is reduced and viscosity slightly increased. Retreat saves the plasmodium from death. The tolerance of slime mold protoplasm for radium radiation is therefore very great.

12. Electricity.

The effect of electricity on protoplasm has been extensively studied with a great variety of results. If the voltage is high enough, a volt or two, coagulation and death result. Sometimes there is an interesting orientation of protoplasmic particles into parallel rows.

Velten (202) and Hörman (89) found the protoplasm of *Nitella* to be surprisingly sensitive to electricity, only 0.000,000,031 ampere being sufficient to cause temporary stoppage of flow.

Amperage alone is of little meaning. It is current, *i.e.,* voltage and amperage, which is effective.

Watanabe *et al.* (213) found the plasmodia of several species of Myxomycetes to show galvanotaxis, *i.e.,* there was locomotion of the plasmodium to the cathode. It was most pronounced at 6–10 volts and 0.15–0.27 mA. Winer and Moore (221) say that a plasmodium (*Physarum*) shows negative galvanotaxis. Kôketsu's (110) extensive studies yielded positive effects of an electric current on protoplasm. He measured the speed of streaming after repeated electrical shocks.

Heilbrunn and Daugherty (83) applied an electric current to *Amoeba* and to *Elodea.* They kept *Elodea* leaves in darkness and observed the movement of chloroplasts toward the cathode which

led them to believe that the electric charge on protoplasmic inclusions is positive under normal conditions. When the material was illuminated, *i.e.*, during photosynthesis, a reversal in charge took place and migration was to the anode. This is a remarkable observation and should be checked on a variety of material.

13. Hydrostatic pressure.

Marsland (124, 125) has made a thorough study of the effect of hydrostatic pressure on protoplasmic flow and finds that with increasing pressure the rate of streaming in cells of *Elodea canadensis* diminishes regularly. Complete abolition of streaming occurs at about 400 atmospheres. This effect is freely reversible, provided the higher degrees of compression (above 400 atmospheres) are not maintained for more than half an hour. When the decompression is by steps, streaming is resumed within one or two minutes of partial decompression. The retardation of streaming appears to be closely related to changes which pressure induces in protoplasmic consistency, as measured by the centrifuge method. The interpretation of these results is based on the proposal that cyclosis is motivated by a cycle of sol-gel reactions and consequently is a phenomenon fundamentally related to amoeboid movement.

If I may object to one deduction based on this excellent work by Marsland, it is that change in viscosity may not be the cause of retardation of streaming. So tremendous a pressure is quite likely to upset the mechanism of flow and therefore alter the motive force. Also, although sol-gel reactions undoubtedly take place, they are, in my (178) belief, incidental, for it is contractility, not viscosity changes, which motivates protoplasm (XII–11).

14. Shock.

Workers in microdissection have long known that sudden intrusion of a needle into protoplasm will often stop streaming. It seems likely, therefore, that any shock may be the cause of temporary suspension of movement.

In order to test this hypothesis more methodically, Epstein and I (136) carried out experiments involving shock produced by the fall of a drop of water. When the flowing protoplasm of slime molds is struck by a falling drop of water, all movement at and near the point of impact stops, if the height of fall is great enough. The protoplasm at a distance from the point of impact continues to flow

normally. The duration of quiet or "shock anesthesia" increases with the distance through which the drop falls, and is further dependent upon physiological factors, such as vigor, age and development of the plasmodium. Younger portions recover sooner than older ones. A drop falling from a height of 15 cm. will produce "shock anesthesia" for $1\frac{1}{4}$ minutes in thin peripheral protoplasm, whereas thicker channels may remain in a state of anesthesia for four minutes. Vibrations of low frequency applied to the protoplasm of slime molds in such a way as to produce direct pressure on the protoplasm bring about pronounced changes in the physical state of the protoplasm, but when vibrations are applied laterally to the cover-slip, without direct contact with and therefore without pressure on the plasmodium, then no effect is observed. Neither streaming nor, apparently, the physiological state of the protoplasm, is in any way affected. That the protoplasm of slime molds shows no pathological change whatever when shaken at the rate of 120 times a second, through an amplitude of 0.1 mm., is rather surprising. Evidently it is the kind of shock which determines in great measure the kind of physiological response.

15. Supersonic waves.

During the still brief period over which the effects of supersonic waves on colloidal systems and living organisms have been studied (222), little reference has been made to their influence on protoplasmic streaming. However, some there is; thus, Yagi (223) states that rotation in *Elodea* can be stopped or induced by extremely weak ultrasonics. He adds that if *Elodea* cells are subjected to ultrasonics for a long period of time, they are plasmolyzed and the whole protoplast rotates.

16. Centrifugal force.

The effect of centrifugal force on living organisms has received considerable attention. It seems to have little effect on microscopic organisms, such as bacteria, but can be highly damaging to larger cells, particularly when these are in a very susceptible condition, as during mitosis. The influence of centrifugal force on streaming of protoplasm has, so far as I am aware, not been studied. Attention is called to this problem here to point out the need of such experiments.

17. Gravity.

Ewart (59) concluded that gravity had no influence on protoplasmic streaming. Vouk (209) likewise found that gravity has no effect whatever on either the direction of protoplasmic flow or its rhythm. That a plasmodium shows no geotaxis was also the opinion of Stahl (184) and others, but Hofmeister (87) and Rosanoff (155) thought otherwise though without convincing experimental proof.

IX. MOTIVE FORCE

1. Seat.

Various ideas have been expressed as to the place where the motive force responsible for streaming is located. In certain hypotheses on the mechanism of streaming the place where the energy is liberated is obvious; thus, if surface tension is responsible, the outer layer of the protoplast becomes the seat of the motive force.

Jahn (92) placed the motive force in the granular plasm of slime molds, whereas de Bary (10, 11) did just the opposite and put it in the hyaloplasm or ground substance. DeBary's viewpoint is more likely the correct one, though there may be no difference of opinion here, for hyaloplasm is ground substance. Only when the hyaloplasm forms the outer hyaline layer of a protoplast layer, as in Myxomycetes, does it become morphologically distinguishable from the inner granular substance. Then may its position at the surface give it specific physiological qualities.

If de Bary had in mind the outer hyaline layer of a protoplast rather than the hyaloplasm which constitutes the matrix of the inner granular plasm, then his hypothesis is especially significant in the light of some recent theories based on contractility (Section XII–11). The hyaloplasmic layer in slime molds retains its individuality in some as yet unknown way. It is a remarkable and as yet unexplained fact that the hyaline layer at the periphery of plasmodia stays free of granules. This is not due (35) to the thinness of the wedge formed by the peripheral protoplasm, for it is possible to focus on an appreciable layer of protoplasm beyond the front line particles. Furthermore, were the thinness of the hyaline layer responsible for keeping out granules, then the last line of granules would not contain, as it does, particles of every size. There would instead be a gradual decrease in size. The fact that the outer line of granules is maintained not because of an anatomical barrier lends

support to the hypothesis that the peripherally located hyaloplasmic layer is physiologically distinct from the inner granular plasm. But this is not in itself sufficient evidence that the motive force responsible for protoplasmic streaming is located at the surface, though numerous investigators think that it is.

Went (218) has outdone all theories of surface mechanisms by placing the seat of the motive force responsible for protoplasmic flow not merely at the periphery but outside the protoplast. Though he at one point says that the seat of the force is "at the outer protoplasmic layer", he elsewhere states that the semipermeable membrane is located "between cell contents and motor mechanism", and adds that this explains why a change within the cell does not influence streaming, whereas the external pH does so readily. "It also explains why the effect of applied auxin is almost immediate, since it does not have to penetrate into the cell". Just how a substance, which has not entered a cell can influence streaming, is difficult to say.

Aside from the fact that Went's hypothesis is very difficult to comprehend, all evidence precludes placing the seat of the motive force between protoplast and wall, for streaming continues normally in a fully plasmolyzed protoplast; indeed, it continues in an isolated protoplast wholly out of the cell (147). This alone is sufficient to show that the interface between protoplasm and wall cannot provide the mechanism for streaming.

The important observation that streaming continues after plasmolysis, has been known since the time of Hofmeister (87) and Velten (201). It was recently rediscovered by Plowe (147).

There are many reasons for believing that the seat of the motive force responsible for protoplasmic streaming is in the cortical layer of a protoplast, but I prefer regarding it as only partly there, or only in certain instances. For example, when a muscle contracts it is not only the surface which is contractile but the entire muscle. So it is in the main, with a protoplast.

Studies on contractility of protoplasm in Myxomycetes (178) have convinced me that we are dealing here not with one unit but with many units, with many centers of activity. Hilton (84), apparently, was the only one in the past to lay emphasis on the presence of several contractile centers in a plasmodium. Meyer (132) expressed a similar opinion when he said that the seat of the

motive force is not at the surface but throughout the cytoplasm. Hilton states that the main currents in the principal "veins" of a plasmodium always, in their alternations, radiate from and converge toward more or less definite centers consisting of larger or smaller aggregations of plasm.

The concept of scattered centers of activity is a most important one. It probably does not apply so fully to uninucleate tissue cells where the motive force responsible for protoplasmic flow may reside in the periphery, but it does apply to gymnoplasts such as plasmodia where the seat of the motive force may be in part at the surface but is also distributed at various points throughout the plasmodium.

2. Energy source.

The vital mechanism responsible for protoplasmic streaming involves the source of energy, the mechanism itself, and the means of transference. Of the last, *i.e.,* how the energy is utilized, nothing is known. Of the mechanism itself, many suggestions have been ventured (Section XII). Our present concern is the nature of the source of energy, which may be considered as to kind and amount.

Historically, there is very little to which to refer. Theoretical estimations of the amount of energy consumed were made by Ewart (59). He applied Poiseuille's law to protoplasmic flow, but as he could not have had a value for viscosity and therefore no knowledge of the work done by the cell, the energy needed to overcome it could not be calculated. His assumptions were also in part faulty. Nevertheless, Ewart estimated that the energy used by a streaming cell of *Nitella* represents a theoretical consumption of 1/200,000 of a gram of cane sugar per annum per gram of plasma moving at the rate of 2 mm. per minute in a cell of 0.4 mm. radius.

Pfeffer (144) assumed that in plant tissues a tenth of the total energy of respiration is consumed in producing protoplasmic streaming.

Most workers on protoplasm have assumed that respiration is the source of energy for protoplasmic streaming (54, 192). Few, however, are aware of the relationship between respiration and streaming. That the energy utilized for streaming is liberated by respiratory processes, is indicated by the fact that streaming, like respiration, is controlled by oxygen, sugar and temperature (22, 23). The quantitative relationship known to exist between stream-

ing and respiration is likewise proof that the one process is intimately associated with the other (51). Furthermore, some experimentally induced changes which influence the rate of respiration in a cell also affect protoplasmic streaming (54, 194).

The development of adequate methods to measure respiration in very small pieces of tissue has made it possible to demonstrate a close relationship between it and protoplasmic streaming. The micro-volumeter of Fenn (61) and the polarographic micro-respirometer of duBuy and Olson (54) permit measurements of respiration in the same sample of tissue in which streaming has been determined.

Work by duBuy and Olson (54), Sweeney and Thimann (194) and Bonner (19) has led to the assumption that there are at least two respiratory systems, one of which is concerned in supplying energy for streaming. Thimann is of the opinion that when streaming ceases in leaves kept under water without stirring, the "bulk" respiration consumes oxygen so rapidly that none is available for "the special streaming respiration". Hence, any agent which will reduce total respiration will allow oxygen to become available for the streaming system. Histidine was shown by Bonner (19) to have this effect; it reduces total respiration in the oat coleoptile.

The strict dependence of streaming on oxygen has led all workers to regard respiration as the source of energy for protoplasmic flow. Clark (41, 42) found streaming in all types of cells to be very sensitive to reduced oxygen pressure.

Bottelier (23) demonstrated that protoplasmic streaming gradually comes to a stop in tissue which is covered with a coverglass. Woods and duBuy (In press) revealed that this stoppage occurs in stages. Renewal of the water causes instantaneous increase in streaming to the original rate because of renewal of the oxygen supply. After prolonged suffocation, both streaming and respiration stop. The process is reversible if the total suffocation has not lasted too long (30 min. or more).

DuBuy (51) has obtained comparable respiratory and streaming data for the tobacco leaf. Respiration rates for a section of tobacco leaf are 95, 65 and 50 micro-liters per hour; and the corresponding streaming rates are 11–15 μ, 6–8 μ and 4–6 μ per sec.

Further evidence of a relationship between respiration and streaming is had in the work of Olson and duBuy (140). They found that there is a parallelism in the effects of respiratory poisons

on the two processes. A solution of 0.01 N KCN applied to an oat coleoptile decreases the streaming rate 50%. The same concentration decreases the respiratory rate from 4.4 to 2.88 micro-liter per hour or to 61% of the original rate. The authors conclude that these and similar data show that there are at least two respiratory systems. One is governed by non-cyanide-sensitive enzymes and the other by cyanide-sensitive enzymes, presumably the cytochrome-oxidase-non-iron-containing yellow ferment system. The data show that protoplasmic streaming rate is affected quantitatively by the same specific respiratory poisons as is respiration rate. This demonstrates a direct dependence of protoplasmic streaming on respiration, and thus a dependence of streaming on the two respiratory systems which contribute to the overall respiration rate. Thus may streaming rate be used as an index of cellular respiration.

Those who have attempted to correlate streaming with respiration are all of one opinion as to the existence of two respiratory systems, but there is difference of opinion on whether one or both of these are associated with streaming. Thimann (192) believes that of the two respiratory systems only one is intimately associated with streaming. Thus, dinitrophenol reduces streaming, although it is known to accelerate total respiration. It, therefore, depresses but one of the respiratory systems and stimulates the other. DuBuy (51), on the other hand, states that the dependence of streaming on both KCN-sensitive and KCN-insensitive respiratory systems proves that the relationship is to one process only.

For some time I have wanted to establish a correlation between respiration and streaming in myxomycetes. It has been shown that O_2 consumption is intimately associated with streaming in higher plants. I (177) had demonstrated that streaming in the plasmodium of *Physarum* is not sensitive to low oxygen pressure, for at a greatly reduced O_2 tension streaming continues unabated. This suggested that the plasmodium is anaerobic.

When the opportunity arose to carry out work on the respiration of *Physarum,* I asked my student, Winthrop Price, to establish, if possible, whether or not high O_2 consumption was necessary for streaming, my own work having indicated that it was not. The experiments indicated that the slime mold *Physarum* is aerobic with an extraordinarily high O_2 consumption, a QO_2 of over 20, yet the oxygen supply may be reduced to a very low concentration

without streaming being noticeably affected. At only 0.5% oxygen streaming still continues, though respiration is cut 99%.

Just as remarkable is the fact that $\frac{M}{100}$ potassium cyanide suppresses O_2 consumption 80%, yet does not stop protoplasmic flow. A concentration of cyanide sufficient to affect flow causes death.

These results may be interpreted on the basis of the postulate that there are two systems (190); one, the primary respiratory system, is responsible for the release of energy consumed in general metabolic processes, and the other, a secondary fermentation process, from which comes the energy responsible for streaming. The former is aerobic with high oxygen consumption, and the latter, though not wholly anaerobic, requires but a very low concentration of oxygen. Thus does the presence of two, possibly independent systems explain a normally high O_2 uptake on the one hand, and continued active streaming at a very low O_2 supply on the other hand.

In placing the source of energy responsible for protoplasmic streaming in respiratory processes, we thereby either ignore other oxidative reactions or include them all under respiration. There are those who regard all cellular reactions involving molecular oxygen with the liberation of energy, whatever the substrate, as respiration, but I cannot agree with this. It is true that the chief function of respiration is liberation of energy, and it is also true that the nature of the substrate, whether sugar, fat or protein is immaterial, but there is one fundamental difference between respiration and other oxidative processes, namely, reversibility. The chain of reactions which constitutes respiration in the strict sense is irreversible, whereas other oxidations are coupled with the reversal of specific processes; thus, in muscle there are reversible anaerobic reactions involving lactic acid formation and splitting of creatinphosphoric acid. These are peculiarities of muscle. It is not the substrate, not phosphocreatin, which is significant, but reversibility of the reaction. The mechanism of oxidation is essentially the same in all cells, but in certain tissues, such as muscle, it is coupled with synthesis in accordance with the special function of the reaction, and synthesis is not a characteristic of respiration.

3. Measurements.

Kamiya (100, 101) has developed a method by means of which

the force responsible for protoplasmic streaming can be measured. A Myxomycete plasmodium consisting of two protoplasmic globules connected by a strand of protoplasm is placed in a double-chamber. The two masses of protoplasm are in different compartments but are connected by a single protoplasmic strand which passes through the agar wall. Construction of the chamber is such that the compartments may be kept air-tight without interfering with the protoplasmic flow in the connecting strand. One of the two compartments is kept at atmospheric pressure, whereas pressure in the other compartment is under control.

When there is no pressure difference between the two compartments, protoplasmic streaming goes on normally, showing regular reversal in direction of flow. When, however, the air pressure of one compartment is modified, the protoplasmic flow through the connecting capillary is strikingly affected. Kamiya was able to accelerate, retard and reverse the protoplasmic streaming artificially. By applying a precise amount of counter-pressure, it was possible to prevent the protoplasm from flowing in either direction. When the protoplasm is kept quiet by counter-pressure, the motive force generated in the plasmodium is exactly balanced by the pressure applied. This balanced counter-pressure, or "balance-pressure", is equivalent to the absolute value of the vital force responsible for protoplasmic flow.

The balance-pressure, and therefore the motive force, fluctuates in both directions between zero and an average of 15 cm. of water. The maximum absolute value encountered was 30 cm. of water. Generally, the force exerted in one direction is stronger than that exerted in the other direction. This is why the protoplasm, when it is free to move, flows more in one direction than in the reverse, and this is what makes advancement possible. There is thus a rhythmical change in the direction and magnitude of the driving power of protoplasmic flow, with an interval of about 100 seconds.

X. FUNCTION

At least one function of protoplasmic streaming is obvious in those organisms which move by amoeboid or similar activities; but whether or not it has other functions in these organisms and what its function is in organisms where amoeboid locomotion is lacking, is unknown.

1. Locomotion.

In slime molds and amoebae there is little doubt that streaming is primarily responsible for locomotion. Mast (128) believes that the main and possibly the only function of protoplasmic flow in *Amoeba* concerns locomotion. How it functions will be discussed later.

2. Food transport.

Distribution of nutritive matter is an often cited function of protoplasmic streaming. If distribution of nutrition is not the only nor the chief purpose of protoplasmic flow, streaming, nevertheless, brings about such distribution. It is idle to deny this. Other means of food transport may be and undoubtedly are present, but streaming augments them. Bierberg (18) and Van den Honert (88) discuss the problem.

Hartig (79), who first noted streaming protoplasm in woody tissues, suggested that its function might be the moving of nutritive sap. DeVries (211) also suggested that the function of protoplasmic streaming might be the moving of nutritive matter. He noted that the diffusion of ions is too slow to account for rapid translocation of food stuffs in the plant.

Grehn (77) likewise thought food transport the chief function of mass streaming in Phycomycetes. Pfeffer (144), on the contrary, citing work by Ewart (60), said that in ordinary plant cells transference of nutrient matter by diffusion is more rapid than by streaming. Brown and Escomb (27), Curtis (50) and others believe, on the other hand, that protoplasmic streaming does function in translocation of solutes. Strasburger (186) discarded the hypothesis because streaming could not be seen in phloem sieve tubes at the age at which they are most active in transport. The inability of Strasburger to see streaming in phloem, Curtis (50) thinks was due to injury in preparing the tissue for observation. It is also possible that flow takes place only at times, under favorable conditions.

Mason and Maskell (127) assume that diffusion is the means of translocation of sugar through the sieve tubes of *Gossypium*. The diffusion constant of the sugar in the sieve tubes is much higher than that of sugar in water. These experiments led Van den Honert (88) to demonstrate in a model that diffusion of substances

can take place by surface activity along the interface of two substances; this, in a way, correlates translocation and streaming.

An important matter in food transport by streaming is the getting of the nutrient from cell to cell. Diffusion would accomplish this through cellulose walls, but streaming could only if intercellular protoplasmic connections exist. Existence of intercellular protoplasmic threads or plasmodesmata was long questioned, indeed, emphatically denied, but they are coming back into their own again and now have rather general scientific recognition. (Section VII–4.)

3. Aeration.

Aeration or oxygen distribution is another possible function of protoplasmic streaming. Again it can be said that whether or not aeration is a purpose of flow is unknown, but it certainly is a result of flow.

4. Growth.

That protoplasmic streaming is correlated with if not in some instances the cause of growth is an obvious deduction. The flow of protoplasm into young sporangia of bread mold or into the terminal portions of *Vaucheria* hyphae would almost certainly tend to expand the young walls and thus bring about extension or elongation of the young growing region. In such cases protoplasmic streaming is directly responsible for enlargement. But there are numerous possible indirect correlations between streaming and growth; thus, Thimann (192) states that in the coleoptile growth and streaming are inhibited by indolacetate with little or no decrease in respiration.

Buller (29), from his studies on fungi, concludes that as flow of protoplasm in the mycelium of *Fimetaria* is in one direction only, from older hyphae toward rapidly growing younger hyphae without any reversal, there can be no doubt that this translocation serves to aid the younger hyphae in their growth.

Bottelier (21) demonstrated that protoplasmic streaming in oat coleoptiles provides a micromethod for demonstration of changes in cell metabolism. DuBuy and Nuernbergk (52) discussed the possibilities which this method offers for explanation of various problems of transport in oat coleoptiles, *e.g.*, light-growth reactions, phototropic curvatures and effect of temperature.

DuBuy and Olson (54) correlate streaming, electric potential, food transport and growth, and thus regard streaming as directly, even though only partially, responsible for growth.

5. Reproduction.

Reproduction, whether of cells or organisms, may involve protoplasmic streaming as a primary factor or as an incidental feature. In higher plants reproduction consists of fusion of two nuclei, and bringing the two nuclei together is an important feature of the procedure. It has been suggested that the active streaming of protoplasm in pollen tubes serves to transport the male nucleus down the tube from pollen grain to ovule.

Protoplasmic streaming may play a part in cell division but it may also prove a hindrance. There have been a few speculations on the relationships between protoplasmic flow and fission or mitosis. One of these is that by Spek (182) who advanced the hypothesis that cytoplasmic currents flowing toward the centrosomes lead to production of high surface tension at the equator of the egg bringing about furrowing.

Streaming of the protoplasmic matrix during midmitosis has been observed in animal eggs. At midmitosis in, *e.g.*, an echinoderm egg, there is centripedal flow of material from the gelated cortical protoplasm, which forms the asters, toward and into the center of the spindle region. This movement of fluid from the surrounding protoplasmic jelly into the hyaline spindle region is probably not streaming, as usually understood, but a process comparable to syneresis (173).

6. Arrangement of cell parts.

Arrangement of cell parts must in large measure be due to streaming protoplasm. Whereas the most obvious and easiest way to do a thing is not always the way it is done, yet it is certainly not only a possible but a likely way. The casual here and there movement of nucleus, small vacuoles and fat droplets in a cell is surely accomplished by flowing protoplasm; and the central position of the large vacuole in a typical plant cell is due to peripheral rotation of the protoplasm. In short, where the position of cell parts is a chance one, it is determined by moving protoplasm. But where orientation is functional, as in chloroplasts, the mechanism of move-

ment is still an unsettled question, though streaming may be responsible.

7. Building of the cell wall.

Orientation of the micellae which constitute the cell walls of plants (134) is similar to that of bricks in a wall (173). These structural units are in perfect alignment, running parallel to the axis of the cell. It is particularly significant that the micellae in transverse walls are parallel to the axis of those walls and therefore at right angles to the micellae in longitudinal walls.

Cellulose molecules are long fibers. If these—molecules, not micellae—are carried by the flowing protoplasm which lays down the wall, they will be deposited with their molecular axes parallel to the axis of the wall. In such a way may the arrangement of micellae in the cell wall be attributed to streaming protoplasm.

8. Spiral structure.

Among the earliest observations on streaming was that of Amici (3, 4) who, seeing the spiral flow of protoplasm, attributed it to a twist in the cell walls. Many plant structures are spiral. Castle (36) says the direction of protoplasmic streaming determines the orientation of spiral structures in plant cells. The same situation holds here as in the preceding paragraph, that is to say, if streaming protoplasm contains long polysaccharide chains, these will tend to become aligned in the direction of streaming. If the wall grows in thickness by addition, apposition, of these chains, the orientation existing at the time of attachment may be preserved in the wall.

Spirality is of common occurrence in animate nature. It exists in wood, in cotton fibers, in the primary and secondary walls of cells, in trees, chromosomes, *etc.*, and Chadefaud (38) attributes it to protoplasm. He believes there is a helical torsion to the cytoplasm of *Draparnaldia;* in fact, he says that all within the cell—cytoplasm, chromosomes, filamentous chondriosomes, lipoid globules and granules—arrange themselves in an elongated helical coil. This torsion could not exist in the cytoplasm were it not supported by a relatively rigid skeletal structure, which is, however, invisible. Quite a number of other algae show the same helical orientation of cytoplasm.

From such observations it would appear that the path of protoplasmic flow is likewise helical, and that spirality in streaming is of more general occurrence than appreciated.

Hörmann (89) concludes that spiral streaming in *Chara* and *Nitella* is an adaptation to favor translocation.

9. Diploidization.

When a haploid mycelium is diploidized by another of the opposite "sex", the nuclei of the one must pass from cell to cell along the hyphae of the mycelium which is being diploidized. In considering this problem, Buller (29) concludes that previous observers have been mistaken: there is no breaking down of the walls to permit passage of nuclei, but in the middle of each septum of Ascomycetes there is an open pore through which the nuclei pass. As the movement of nuclei is a passive one, diploidization is added as another function of streaming.

XI. RHYTHM

Protoplasmic streaming often shows a remarkably consistent rhythm which is at times obvious and pronounced, at other times feeble, and sometimes apparently lacking. In slime molds the protoplasm flows for a time in one direction and then reverses its course. This characteristic feature of protoplasmic movement in Myxomycetes was noted by de Bary in 1859 (10), Cienkowski (40) and Hofmeister (87), and others have referred to it; Vouk (210) made a thorough study of it.

Among those who carefully studied the rhythm in flow in slime molds is Vouk (210) whose experiments led to formulation of the following "laws": *a.*) The sum of the durations of progressive and regressive flow is a constant. *b.*) All main lines of flow in one plasmodium have approximately the same rhythmic period. *c.*) The period increases with age and size of the plasmodium until a certain maximum is reached which remains constant until fruiting. The first two conclusions of Vouk are true only in part and under ideal conditions.

Vouk's assumption that the sum of the durations of progressive and regressive flow is a constant is true, provided the numerous disturbances, some of which are the result of changes in the external environment and some internal interference phenomena, are all neglected.

My own work gives no support to Vouk's third conclusion that protoplasmic rhythm changes with age and size of the plasmodium (See Section VI–4).

In his second paper Vouk (211) reaches the conclusion that progressive and regressive streaming in slime molds indicates polarity of the plasmodium. When several fans of pseudopodia, toward which the progressive stream flows, are attached to one common base, toward which the regressive stream flows, the plasmodium is then said to be multipolar. Vouk further established the following relationships. In interpreting these, Vouk's use of the word "amplitude" must be clarified, for he uses the term erroneously. He does not mean one-half of a wave height, but the distance traversed in one direction. He concludes that the "amplitude" of the progressive stream is greater than that of the regressive, which means that the time of flow is directly proportional to "amplitude": that the velocity is inversely proportional to the rhythmic period: and that the rhythmic period is directly proportional to the "amplitude" and inversely proportional to velocity.

The most precise study of the mechanics of rhythmic protoplasmic flow yet made is that of Kamiya (100, 101). His experiments, already referred to, involve opposing protoplasmic flow with air pressure. Two small pieces of slime mold plasmodia are placed in separate compartments of a small chamber, but remain connected by a fine protoplasmic strand which passes through and is sealed in a wall of agar. The pressure of air which is exactly sufficient to hold the protoplasm quiet, *i.e.,* the "balance-pressure", is a measure of the vital motive force responsible for streaming. The undulating curves, or dynamoplasmograms obtained show highly interesting patterns which differ from specimen to specimen and from time to time in the same specimen. Kamiya concluded that these divergencies in patterns are due to interference effects. His conclusion is based upon a theoretical consideration of dynamoplasmogram patterns which, on analysis, are found to consist of a series of simple harmonic components, from which Kamiya concludes that protoplasmic flow in Myxomycete plasmodia is governed by a number of component rhythms having different frequencies and magnitudes; in short, a plasmodium is a polyrhythmic system.

One further discovery made by Kamiya (101) is particularly important to the problem of rhythm in life processes. He found that the balance-pressure necessary to hold protoplasm quiet varied rhythmically even when the protoplasm continued to flow in the same direction for a long period of time. An observer of proto-

plasmic flow in slime molds would maintain, indeed on more than one occasion it has been maintained, that as streaming often continues for an excessively long time in one direction, periodicity in flow is not always present, is not perfectly rhythmic, and therefore not basic. The work of Kamiya demonstrates that any opposition to rhythm in life when based on apparent and occasional absence of rhythm, or on ability to experimentally disturb it, is not justified. The motive force or mechanism responsible for periodic movement maintains its rhythm even though that rhythm is not always made manifest by periodic reversal in direction of flow. A rhythm may thus be present even though there is no outward evidence of it; in short, in animate nature when no rhythm is observed, it may actually be present but not obvious. If a property of matter appears general, its occasional absence is more likely apparent than real.

The universal existence of rhythm in animate and inanimate nature is an unsettled problem in the minds of some scientists. There are those who view it as mythical, or, if real, determined solely by environment and therefore a superficial and variable property. I view the subject in another light; likewise Schimper who regarded rhythm in a plant's activities as an innate heritable property. The fallacy in the reasoning of those who view rhythm in life as superficial is the faulty belief that ability to disturb physiological rhythm is proof of its superficial nature. The true situation may be expressed in this way: for any given character to appear there must be both a genetic background and a suitable environment. A character is the product of a particular genetic makeup developing in a particular environment. Some characters are rigid and mature in a great variety of environments; others are feeble and readily suppressed or diverted. Reproductive cycles show considerable variation in stability. A two-week sporulation rhythm in slime molds (180) held rigidly over a period of four months, whereas before that period no fruiting whatever had been observed for two years. Other reproductive cycles are more firmly established, as is true of heat in animals, yet even this rhythm may be disturbed. Is it therefore less real and less fundamental? And will the time yet come when a biologist will search for a three-quarter minute rhythm in the environment of his laboratory, or in the climate of a woodland, in order to account for the three-quarter minute rhythm in the pro-

gressive and regressive flow of slime mold protoplasm? There can be no question of the reality and innate character of rhythm in protoplasmic streaming of certain organisms, a rhythm visible and convincing to the most skeptic observer, and one that will probably defy all attempts to correlate it with environmental factors. Drastic changes in environment will disturb it, but the rhythm is not occasioned by environment.

The work of Kamiya leaves no doubt as to the reality and significance of rhythm in protoplasmic flow. The occasional and inexplicable opposition to rhythm in animate nature, in growth, reproduction and protoplasmic streaming, must collapse under such precise determinations of a process so fundamental as protoplasmic movement. The rhythm in protoplasmic activities is the basis of the rhythm in gross processes such as growth of an organism as a whole.

XII. MECHANISM OF PROTOPLASMIC STREAMING

1. Surface tension.

Few biological phenomena have escaped interpretation in terms of surface tension.

The rôle of surface forces throughout nature can hardly be overestimated, yet undue emphasis has been given the part played by them in certain biological phenomena. That changes in surface tension occur in active protoplasm is certainly true, but there is no experimental evidence that these changes produce flow. Until some such concrete evidence is presented, the theory that surface tension is the cause of protoplasmic streaming must remain in abeyance. However, the surface tension hypothesis is not without possibilities. Every interface within protoplasm is the seat of surface forces (114).

When Pfeffer (144) and Ewart (59) say that the energy of movement is not liberated at the boundary, it may yet be true that the energy of protoplasmic flow resides at many boundaries within the cell. In any opposition to a surface hypothesis of protoplasmic streaming it must be borne in mind that, as Harvey (80) states, there is a distinction between surface tension and tension at the surface.

The chief objection to surface tension theories in biology is the difficulty of doing anything with them. Those who advance such theories rarely interpret the mechanism.

Tiegs (196) has summarized much of the work done on the relationship between surface tension and biological phenomena, with especial reference to amoeboid movement and muscular contraction.

2. Hydration.

Water will flow up a wick if the top end is in air and the bottom end in water. As protoplasm is mostly water it would probably move along a strand if there is dehydration at one end and hydration at the other. Another analogy is the ascent of sap, which is said to rise because of dehydration by transpiration at the leaves and hydration by osmosis at the roots. I reject the hydration theory of protoplasmic flow not so much because of inherent mechanical difficulties, though the hyphal tube of *Rhizopus* is no wick, but because many types of flow, such as the rotational movement of the protoplasm in an *Elodea* cell, do not fit into the hydration picture at all, and because in those instances where the hydration theory is in a sense mechanically feasible it does not apply.

When protoplasm is flowing up the hyphae of *Rhizopus* which are imbedded in a moist substratum, toward and into young aerial sporangia, one can readily understand how hydration and dehydration may have an effect; but when that same protoplasm reverses its direction of flow, and when the protoplasm in a wholly submerged hypha continues to flow, then it is evident that hydration and dehydration are not the determining factors. In saying this it is not implied that these factors do not influence streaming. They do, as Andrews (7) has shown. He states that flow toward the exposed tip may be induced by passing dry air over a culture of bread-mold, the hyphae of which protrude from a moist substratum, and when saturated air is admitted, flow from the tip takes place. Andrews' experiments force one to recognize that transpiration influences protoplasmic streaming; it may induce, accelerate or retard it, but it is not the cause of the movement, for flow continues regardless of transpiration and in direct opposition to it. In justice to Andrews let it be said that he was fully aware, as he states, that streaming takes place in either direction in a water-saturated atmosphere.

3. Osmosis.

Osmosis, like hydration, is a force which has been held responsible for protoplasmic streaming. It is certainly a force influencing flow.

Voronin (208) was apparently the first to suggest that turgor is the cause of protoplasmic movement. He was followed by Ternetz (191), Grehn (77) and Jahn (92). Jurišić (95, 96) and Lauterbach (117) have accepted the theory.

Grehn (77) adds his support to the osmotic theory of protoplasmic flow by stating that in Phycomycetes transpiration at one part and endosmosis elsewhere account for streaming. Like the transpiration hypothesis of the ascent of sap which fits northern climates but can hardly function in a saturated tropical atmosphere, hydration and osmosis can be regarded as the causes of protoplasmic streaming only when all conditions are favorable, which is often not the case.

Andrews (7) believes transpiration to be the cause of streaming, from which it follows that osmosis is also a factor.

Schröter (162) states the matter very definitely: "Protoplasmic streaming in *Mucor* and *Phycomyces* rests on osmotic and transpiration forces". He and Ternetz (191) believe to have proved the foregoing when they experimentally showed that streaming in *Mucor* and *Ascophanus* takes place toward the point where concentrated solutions of osmotically active substances are applied. Apparently, then protoplasmic streaming is simply the osmotic movement of water toward a solution of higher concentration. There can be no questioning of the fact that osmosis affects streaming, and there is no denying the experiments of Andrews, Schröter and Ternetz, but the conclusions they draw rest on a very common fallacy, that of regarding any factor capable of modifying or upsetting a mechanism as the cause of or even involved in the normal functioning of the mechanism.

Andrews (7) states that if streaming is due to osmosis there can be no peripheral movement in the opposite direction, as stated and figured by Schröter (162), which confirms the statement of Ternetz (191). But there usually is a return stream in mucors.

4. Sol-gel reversibility.

Mast (128) advanced a sol-gel reversibility hypothesis of amoeboid movement which has been well received. As a mechanism of flow it has been applied to protoplasmic streaming on the assumption that the two phenomena are indistinguishable.

The sol-gel hypothesis of amoeboid movement, as given by Mast (128), is as follows: The amoeba consists of three primary regions,

the outer plasmalemma, the cortical plasmagel and the inner plasmasol. Four primary processes are involved in locomotion; attachment to the substratum, gelation of the plasmasol at the anterior end, solation of the plasmagel at the posterior end, and contraction of the plasmagel at the posterior end. Gelation of the plasmasol at the anterior end extends the plasmagel tube forward as rapidly as it is broken down at the posterior end by solation. Contraction of the plasmagel at the posterior end drives the plasmasol forward.

If one distinguishes between amoeboid movement, the locomotion of slime molds, and protoplasmic streaming, then the part played by sol-gel transformation in amoeboid movement is beyond the province of this paper, unless it also plays a part in protoplasmic streaming. In my opinion (178) the mechanism of protoplasmic streaming is not one of sol-gel reversibility. A number of able workers disagree with me on this, and they have carried over to Myxomycetes ideas which were developed from studies on *Amoeba*. *Amoeba* and slime molds have much in common but the mechanisms of their locomotion are not necessarily identical. This conclusion may be questioned, but it is certainly obvious that protoplasmic flow is not the same in Myxomycetes, where streaming is rhythmical with an alternating progressive and regressive flow, and in *Amoeba* where streaming is a continuous movement in one direction with no rhythmical reversal. The mechanism of locomotion in slime molds is similar to that of the incoming tide, in that the medium of both surges forward, then retreats, and surges forward again. This is not the case in amoeboid movement.

The presence of a hyaline layer in slime molds made the carrying over to them of the sol-gel theory of locomotion, as applied to amoebae, very easy, for in slime molds there is the desired cortical gel and the fluid endoplasm on which the theory rests.

Lewis (120, 121) and Marshall (124, 125) agree with Mast (128) in regarding sol-gel reversibility as the mechanism responsible for protoplasmic streaming in amoeboid cells, resulting from localized changes in protoplasmic volume which accompany the sol-gel reactions of active locomotion. Although the magnitude of these volume changes is unknown and must be quite small, it may be that gelation and shrinkage of the sol in the anterior parts of the cell would favor the flow of sol in this direction. Simultaneously, solation of the plasmagel which occurs in the posterior parts of cell, due to the volume increase, would augment the forward flow of the sol.

I cannot help but feel, as I have already stated, that in spite of the careful observations made on the locomotion of *Amoeba,* phagocytes, fibroblasts and slime molds, and the thoughtful interpretations of these observations, it is yet true that the essential feature of the mechanism of protoplasmic streaming is not a sol-gel transformation, but contraction and relaxation. It may well be that sol-gel transformations take place, and they may occur concurrently with streaming, but they are not, I believe, the essential feature of the mechanism of flow. Streaming is occasioned by contractile forces which need not involve a viscosity change or a sol-gel transformation. Contractility is more deeply seated than that. It is molecular in origin (178).

5. Myelin processes.

In a diagrammatic cross section of the cytoplasm of a plant cell, Scarth (158) pictures myelin processes extending from the protoplast. Such processes, or lipoidal papillae, are rather common on the surface of cells, especially echinoderm eggs. Scarth (157) is of the opinion that there is a correlation between myelin processes and protoplasmic streaming, particularly in reference to kinoplasmic streams within the body of the cytoplasm. His idea is that there is an interface between layers of orientated lipoidal molecules which slip smoothly over one another when local differences of surface tension are set up. There is thus in the formation of these layers an analogy to myelin activity.

6. Coacervates.

Introduction of the concept of coacervates by Bungenberg de Jong (30) has led to its use in the interpretation of protoplasmic behavior. Of the kinoplasm Scarth (158) says: "its apparent immiscibility with water points to a coacervate rather than a sol. In a coacervate, according to Bungenberg de Jong, the colloidal units are attracted by electrostatic force but kept apart by that of solvation. Thus the aggregate, while coherent, may be quite fluid. The whole cell has been compared by de Jong to a compound mixed coacervate".

Of the types of coacervate which have so far been studied extensively by Bungenberg de Jong, those which most resemble protoplasm are the auto-coacervates of phosphatides. This correlates coacervate and myelin activity.

Several points of resemblance between protoplasmic and coacervate behavior are listed by Scarth (158), but our interest is primarily in streaming. In this respect the complex coacervate system resembles protoplasm in the ease of slippage of its bimolecular lipoidal films.

7. Autonomous propulsion of particles.

That the visible particles in protoplasm flow under their own power is generally not an accepted view, though Bünning (31) states it is uncertain whether all of the protoplasm flows; possibly only the microscopically visible particles move. After all, the particles are all the indication we have that the protoplasm is flowing. Though the majority opinion opposes this view, it may still be true that some particles, especially the submicroscopic micellae in protoplasm, are capable of self-motion; at least so thinks duBuy (51) who has developed a theory of protoplasmic flow which involves the assumption that ultramicroscopic particles move independently.

In the foregoing section it was stated that complex colloidal coacervates show streaming movements which can be correlated with myelin activity. DuBuy (51) believes that invisible protein particles (colloidal micellae) which carry respiratory enzymes are polar. The respiratory process, catalyzed by the particle (an enzyme-carrier) releases energy which goes into motion in a polar way, comparable with the motion of a rocket. DuBuy offers this "rocket hypothesis" as a working theory for the understanding of the relation between respiratory processes and streaming. He says: "Using the coacervate model of Kruyt and Bungenberg de Jong it might be conceived that the lipoid-protein coacervate carries a protein-respiratory ferment in a polar position. The release of energy during respiration at this pole, by suddenly changing surface tension or by actually releasing molecules, would propel the particle".

The hypothesis of duBuy is certain to meet with criticism if for no other reason than that it is novel and contrary to orthodox views; but he is not alone in regarding the motive force responsible for streaming as resident not in the matrix of protoplasm but in the suspended particles, whether these latter are ultramicroscopic colloidal micellae or visible cell inclusions. Valkanov (199) is of the opinion not only that the movement of nuclei and chromatophores is autonomous but that this movement is responsible for cytoplasmic flow.

8. Kinetic energy.

Meyer (131) advances the plausible but incomplete hypothesis that protoplasmic streaming is caused by the heat motion of molecules. Such a remark is without great meaning unless interpreted. The heat motion of molecules is kinetic energy, and the energy is useless unless harnessed. In short, Meyer states an obvious source of energy but tells nothing of the nature of the mechanism.

9. Magnetism.

Magnetic forces did not escape the attention of the pioneer workers on protoplasm. The cell wall was regarded as magnetically charged. Ewart (59) made studies on the effect of paramagnetism. Magnetism has been resorted to in explanation of the movement of chromosomes and the configuration of the mitotic figure, but all attempts to prove that protoplasm responds to a strong magnetic influence have failed, from Faraday, who had a student hold his head between the poles of a powerful electromagnet, to recent attempts to disrupt the karyokinetic figure by magnetic forces. Apparently, protoplasm is not sensitive to magnetism, but this conclusion may not be the final one, for biologists have failed in their experiments always to distinguish between para- and diamagnetism, and these may affect protoplasm differently. However, as matters now stand, protoplasm is not responsive to magnetic forces; indeed, the very group of pioneer workers (28) who were the first to advance electro-magnetic hypotheses of protoplasmic movement showed that the direction and velocity of streaming are not influenced by a strong magnetic field.

10. Electrical forces.

The tremendous advance made in our knowledge of electrical forces, especially those involving the movement of colloidal particles and of fluids through capillaries, those which Freundlich (72) called "electrokinetic" phenomena, must inevitably have led to electrical hypotheses of protoplasmic behavior. These are further supported by the known electrical properties of some organisms, and measurements of electrical potentials in living systems. Most of this work is new, but the earliest experimenters on protoplasm were not unaware of the possible rôle of electrical forces in life, though they had no knowledge of electrokinetic phenomena as we

now know them. They could, therefore, do little more than suggest that electricity was, or was not, a factor in physiological processes such as protoplasmic streaming. Ewart (59) thought not, whereas Ssawostin (183) believed protoplasmic movement to be electrical in nature.

Among electrokinetic phenomena, that which involves the movement of particles in an electric field is termed electrophoresis or cataphoresis; and that which is the flow of water through capillaries under the influence of an electric potential is electroendosmosis. Protoplasmic streaming has been interpreted in terms of both these phenomena. Whether it is the particles alone (electrophoresis) which move or the dispersion medium (electroendosmosis) which flows, there must exist in either case a difference in potential at the ends of the cell if movement is electrokinetic.

Evidence that differences in potential exist in living systems is now abundant.

Watanabe (215) *et al.* found that the potential of the plasmodium of *Didymium* was always higher (0.1–6.2 mμ) than the surrounding medium of the same pH, and that the front of the plasmodium is of higher potential than the rear. Watanabe (212) *et al.* also established a relationship between direction of protoplasmic streaming and electric potential. The curve depicting change in direction of flow agrees well with that of change in potential except that the waves of the first are slightly in advance of those of the second.

Granting that electrical potential differences exist in living systems, the next question is, do they produce flow. On the assumption that they do, a number of experimenters made measurements, but with little success. Gelfan (74) published positive results. He worked on the alga *Nitella* and was able to measure a potential between the two ends of the long cell. When the direction of streaming reversed, the direction of flow of current also reversed, as indicated by the swing of a galvanometer needle. As streaming slowed down the voltage became less, and when the protoplasm was quiet the potential difference was zero.

Questions immediately arise: is the potential the cause or the result of the streaming; is the potential measured real or set up by the conditions of the experiment? We can only conclude that in any case it is of significance that potentials can be measured in tissue, and it may prove true that they are associated with protoplasmic streaming.

If electrical forces are responsible for protoplasmic streaming, then one thing is certain, that the flow is electroendosmotic and not electrophoretic, for it is the dispersion medium of protoplasm which moves and not the particles alone. The particles are carried along by the medium in which they are suspended. Their movement is a passive one.

M. H. Ross (unpublished results) has cultured slime mold plasmodia on the surface of mercury. Death results within a day or two; but for at least 24 hours there is active growth and movement. The experiment tends to disprove theories of protoplasmic streaming based on potential differences at opposite ends of the plasmodium, because the mercury completely short-circuits the system. One may, however, assume that the potentials reside wholly within the plasmodium and are insulated from the exterior by the surface membrane, or, as the membrane is also protoplasm, by an outer oily film on the membrane.

In discarding electrokinetic theories of protoplasmic streaming, I do not wish to imply that potentials do not play a part in protoplasmic behavior. They undoubtedly do. When porous or selectively permeable membranes are bathed in electrolytes, the membranes are certain to be the seat of electrical forces. Furthermore, all surfaces in living matter, especially those on colloidal particles in suspension, are likely to be charged, and, as such, are responsive to electric influences or may themselves set up an electric field. In short, protoplasm is the seat of innumerable electrical potential differences. Though these are individually very feeble, collectively they may build up a very substantial voltage, as in the ray-fish and electric eel. These potentials may influence protoplasmic streaming, but to what extent and how, we do not know.

11. Contractility.

Contractility is the basis of at once the oldest and newest hypotheses of protoplasmic streaming. It is a property of living matter early noted.

Scarth (157) rightly states that "Streaming is compatible not only with high viscosity but with definite elasticity".

It is strange that so basic a property of protoplasm as contractility should be questioned today when so many biologists (171, 173) from Dujardin (55) to Northen (137), have found protoplasm to be

highly elastic and therefore contractile. It is also a property easily demonstrated.

As far back as 1774 Corti (48), then Heidenhain (82), Reichert (152), Kühne (111), Brücke and Meer (see Hofmeister 87, p. 61), Verworn (204, 205), Vouk (209) and Rashevsky (151) suggested that protoplasmic contractility was responsible for streaming. It was assumed that a wave of contraction passes around the cell, pinching the primordial utricle, and forcing the protoplasm ahead of it as it moves forward. The theory was discarded by de Bary (11) because the contour of the protoplasm toward the cell sap shows no surface waves. De Bary was studying a typical primordial utricle encased in a cellulose wall, where it has so far been impossible to observe the rhythmic motion which the contractility hypothesis demands, probably because the constrictions sought are too fine or too fast for microscopic observation. Pfeffer (144) carried the theory over to Myxomycetes and remarked further that the contractility must be rhythmical to account for the rhythmical reversal in direction of flow which is so characteristic of slime molds.

Failure to find any sign of protoplasmic contractility in dermatoplasts (protoplasts with cell walls, as in *Elodea*) was a temporary setback to the theory of contractility, but once gymnoplasts (such as plasmodia) were studied with the aid of modern cinématography, the sought-for contraction was found (174). It now remains to demonstrate its presence in dermatoplasts.

Pfeffer (144) suggested that protoplasmic streaming in dermatoplasts is basically different from that in gymnoplasts. To this I cannot agree. It is more likely that there is one basic mechanism of protoplasmic flow and not an entirely different principle for each type of cell. To be sure, cells advance by different mechanisms, *Euglena* swims with the aid of cilia, and *Amoeba* moves by a motion of its entire body. It is not inconceivable that protoplasmic flow is accomplished by different means in different cells, but this seems unlikely. The phenomenon is a very fundamental one; it is a potential property of all living matter, and probably rests on one general type of mechanism in all organisms. It is true, however, that rhythmic contractility is readily demonstrated in slime molds and not in the primordial utricle of tissue cells.

If a plasmodium is photographed on a moving picture film every five seconds and shown at the usual rate of 16 per second, thus

speeding up the apparent rate of movement of the slime mold 80 times, the plasmodium is seen to go through rhythmic contractions and expansions which are synchronized with the outward and inward flow of the protoplasm (174).

The mechanism of protoplasmic movement in slime molds is, then, one of rhythmic contraction and relaxation of the plasmodium, with a total of 95 seconds for each pulsation, 45 seconds for systole and 50 seconds for diastole, the additional 5 seconds in time of outward flow account for advancement. Kamiya (101) gives 93.7 seconds for one rhythmic period. Three distinct rhythms in slime molds were first revealed in moving pictures (101). And now Kamiya (101) tells us that the protoplasm of slime molds is a polyrhythmic system, that many rhythmic cycles of the motor mechanism are operative simultaneously in one and the same plasmodium.

The discovery that a plasmodium is a polyrhythmic system would at first thought seem to hopelessly complicate the situation, but actually it is only through a multiplicity of pulsations that it is possible to account for a number of phenomena. One may frequently observe in a plasmodium several lines of flow which cannot possibly be the expression of a single contractile mechanism. There is often a lag in the time of reversal in one strand over that in the other, which means that there is more than one center of contraction in the plasmodium (178).

Another important matter which should be emphasized is that fluid protoplasm is contractile. Usually the contracting regions of a plasmodium are of high viscosity, but protoplasm need not be so to exhibit contractility. The significance of this remark is twofold: it emphasizes that the chief feature of protoplasmic movement is not a sol-gel transformation but the contractility of protoplasm, and that organic colloidal solutions have some of the properties of gels no matter how thin they may be (171).

Protoplasmic contractility has been attributed to a variety of forces. In the case of muscle, change in surface tension was a widely accepted theory. But today it is generally conceded that protoplasmic contractility is due to the same molecular mechanism as are the contractile properties of wool and hair, namely the folded polypeptide chain.

The elasticity of jellies and the high water-holding capacity of thixotropic gels led to the general acceptance of a linear structural

unit. Contractile properties have now led to another feature of the structural unit of elastic organic systems, namely, molecular folding. With it the picture is relatively complete.

The contractility of protoplasm is due to the supercontraction of its principal structural proteins through the folding of molecular fibers symmetrically aligned and joined one to the other by side chains so as to form a three-dimensional lattice. As this is a general conclusion of far reaching significance in biology, it may be restated as follows: Contractility, wherever it occurs in animate nature, whether in the most elementary form of protoplasm or in highly specialized tissue, is due to the shortening of protein fibers by molecular folding. The energy for this work is supplied by the oxidative processes of the cell. There remains the interesting and rather speculative question, whether the rhythm of protoplasmic movement lies in the chemical reaction which supplies the energy, or in the mechanism where the energy is used. If the energy supply is rhythmic, then there must be a periodically reversible chemical reaction in protoplasm. A number of autocyclic rhythmic reactions are known to occur in protoplasm; the glycogen-lactic-acid cycle in muscle is one. A still closer analogy is to be found in the rhythm of nerve conduction where a cycle of oxidation-reduction reactions is assumed to pass along the nerve. The occurrence of autocyclic chemical reactions in protoplasm is not at all unlikely, but it is impossible to say whether the rhythm of protoplasmic flow lies in the source of energy or the mechanism (178).

XIII. STRUCTURAL ORGANIZATION

Scarth (157) emphasizes a very fundamental relationship between the fluid properties of protoplasm and its structural organization. A number of properties of protoplasm rest on what would appear to be a fixed organization of the cell; yet protoplasm flows. Cells exhibit polarity which must be maintained in streaming protoplasm. Physiological organization, metabolic gradients and the like, are not sufficient, says Scarth. The substance composing protoplasmic strands is viscid, extensible and elastic. These are the properties of solids. The impression of fluidity obtained from microscopic observation is in part illusory. There is an architectural organization in protoplasm which is the structural basis of such properties as elasticity and genetic continuity. This organization must be maintained in streaming protoplasm.

Any attempt to arrive at definite conclusions on the architectural features of protoplasm will, in some degree, be highly speculative, but not wholly so. Stereochemists have formulated sound hypotheses of protein and cellulose structures, such as will permit fluidity yet retain certain of the properties of solids (178). Crystallographers have a clear understanding of the organization of liquid crystals. Colloidal chemists are now able to explain double refraction in solutions which show this property when flowing.

Knowledge of the physical properties of liquids which show crystalline and other qualities characteristic of the solid state is sufficient to justify biological application. One of the most significant features of this knowledge is that pertaining to the coexistence of structural continuity of organization, and of fluidity in protoplasm.

The question of retention in protoplasm of an organization and structural continuity maintained during streaming was recently the subject of a chapter in a volume on "The Structure of Protoplasm" (178). I there pointed out the importance of the hydrogen bond as a link in protoplasmic structure. It is a bond one end of which is more feebly attached than the other, thus permitting a ready shift from one atom to another. This permits flow and yet maintains continuity in structure. The hydrogen bond thus provides a mechanism by means of which continuity in structure, elasticity and rapid changes in viscosity of protoplasm may be explained. I conclude that it is truly an encouraging sign in the progress of science when properties of protoplasm such as fluidity, contractility and structural organization, which heretofore were so little understood, can now be interpreted in terms of folded polypeptide fibers, interlocking side chains, hydrogen bonds, and asymmetry of the carbon atom.

XIV. SUMMARY

1. The historical background of work on protoplasmic streaming is presented with Corti (1772) recognized as the discoverer. The significance of streaming is pointed out by indicating its wide distribution.

2. Five types of protoplasmic movement are distinguished: streaming, amoeboid, euglenoid, ciliary and gliding. The possibility of the independent motion of cell parts is considered, discarded in some cases, accepted in others.

3. Five types of protoplasmic flow are enumerated: agitation, rotation, circulation, shuttle and sleeve.

4. A large number of determinations of rate of flow are given, with 0.1 mm. per sec. as a grand average maximum, and 1.25 mm. per sec. as the absolute maximum, the latter in slime molds.

5. That all of protoplasm flows, the matrix carrying the particles, is emphasized.

6. Abnormal types of protoplasmic flow are discussed, both naturally occurring and experimentally induced types. The old idea that the usual form of protoplasmic flow is pathological is pronounced untenable.

7. The influence of internal factors, such as cell form, viscosity and age, are considered. The bearing of viscosity of protoplasmic streaming is fully recognized, but the notion that each and every change in rate of flow, if not indeed every change in any form of cellular activity, is due to a change in protoplasmic viscosity, is pronounced far too simple and hopeful an explanation.

8. The influence of external factors is discussed in detail. Cold and heat both stop flow reversibly. Visible light has little effect. Ultraviolet light has a pronounced effect, but little is known of it. Water has but slight effect except as hydrostatic pressure or shock. Salts have pronounced effects which vary greatly on kind and concentration. Oxygen depletion slows streaming in most cases, but the reduction must be very great to affect flow in slime molds, even though respiration is greatly reduced. Anesthetic agents of proper kinds and concentrations stop flow reversibly and with little or no injury. The influence of numerous other factors is presented.

9. The seat, source of energy, and magnitude of the motive force are considered. The seat may be in the surface layer, but not necessarily so. In slime molds there may be more than one center of activity. The energy is liberated by respiratory processes but probably not by one alone. The magnitude of the motive force is equivalent to an average of 15 and a maximum of 30 cm. of water in slime molds.

10. The function of protoplasmic streaming is probably not one nor the same in all cases. It may be locomotion, food transport, aeration or other life processes, and it is certainly correlated with growth.

11. The rhythmic flow of protoplasm in slime molds is analyzed.

12. Eleven theories of the physical mechanism of protoplasmic streaming are presented, and that of rhythmic contraction and relaxation given preference.

13. The bearing of the structural organization of protoplasm, and of modern chemical concepts of protein fibers, side linkages, and hydrogen bonds, on the mechanism of protoplasmic streaming is presented as an encouraging promise of greater work to come.

* * *

I am indebted to Mr. Noburo Kamiya for assistance in compiling the bibliography.

XV. BIBLIOGRAPHY

1. Alexander, G. The significance of hydrogen ion concentration in the biology of *Euglena gracilis*. Biol. Bull. **61**: 165–184. 1931.
2. Altmann, R. Die Elementarorganism und ihre Beziehungen zu den Zellen. 1890.
3. Amici, G. B. Osservazioni sulla circolazione del succhio nella *Chara*. Mem. Soc. Ital. Moderna **18**: 183–204. 1818.
4. ———. Some accounts of Professor Amici's contributions relative to the motion of sap in plants. Edin. Phil. Jour. **2**: 172–177. 1820.
5. ———. Circulation des Saftes in der *Chara*. Isis 665–677. 1822.
6. ———. Osservazioni microscopiche sopra varie piante. Quart. Jour. Sci. Lit. & Arts **16**: 388–393. 1823.
7. Andrews, F. M. Protoplasmic streaming in *Mucor*. Bull. Torrey Bot. Club **39**: 455–499. 1912.
8. Arthur, J. C. The movement of protoplasm in coencytic hyphae. Ann. Bot. **11**: 491–507. 1897.
9. Balbach, H. Die Wirkung von Harnstoff auf Gymnoplasten. Protoplasma **26**: 192–204. 1936.
10. Bary, A. de. Die Mycetozoen. Ein Beitrag zur Kenntnis der niedersten Thiere. Zeits. Wiss. Zool. **10**: 88–175. 1859.
11. ———. Die Mycetozoen (Schleimpilze). Ein Beitrag zur Kenntniss der niedersten Organismen. 2 Aufl. 1864.
12. Bary, H. A. de and Woronin, M. S. Morphologie und Physiologie der Pilze. II. Entwicklungsgeschichte der *Ascobolus Furcherrimus* Cr. 1866.
13. Becker, Z. E. A comparison between the action of carbonic acid and other acids upon the living cell. Protoplasma **25**: 161–175. 1936.
14. Beikirch, H. Die Abhängigkeit der Protoplasma-Strömung von Licht und Temperatur und ihre Bedingtheit durch andere Factoren. Bot. Arch. **12**: 389–445. 1925.
15. Bělehrádek, J. La viscosité du protoplasma vivant et ses rapports avec l'activité et le vieillissement cellulaires. Publ. Fac. Méd. Brno **3**: 1–28. 1924–25.
16. ———. Temperature and living matter. Protoplasma Monograph. 1935.
17. Berthold, G. Studien über Protoplasmamechanik. 1886.
18. Bierberg, W. Die Bedeutung der Protoplasmarotation für den Stofftransport. Flora **99**: 52–80. 1909.
19. Bonner, J. The growth and respiration of the *Avena* coleoptile. Jour. Gen. Physiol. **20**: 1–11. 1936.
20. Booy, H. L. The protoplasmic membrane as a complex system. Rec. Trav. Bot. Néerl. **37**: 1–75. 1940.
21. Bottelier, H. P. Über den Einfluss des Lichtes auf die Protoplasmaströmung von *Avena*. Kon. Akad. Wet. Amsterdam **36**: 790–794. 1933.
22. ———. Über den Einfluss äusserer Faktoren auf die Protoplasmaströmung in der *Avena*-Koleoptile. Rec. Trav. Bot. Néerl. **31**: 474–582. 1934.

23. ————. Oxygen as limiting factor of the protoplasmic streaming in *Avena* coleoptiles of different ages. Rec. Trav. Bot. Néerl. **32**: 287–292. 1935.
24. Braun, A. Richtungsverhältnisse der Saftströme in den Zellen Characeen. Ber. Preuss Akad. Wiss. Berlin 220–268. 1852.
25. Brown, D. E. S. and Marsland, D. A. The viscosity of *Amoeba* at high hydrostatic pressure. Jour. Cell. Comp. Physiol. **8**: 159–165. 1936.
26. Brown, R. Organs and mode of fecundation in Orchideae and Asclepiadeae. Trans. Linn. Soc. **16**: 685–745. 1831.
27. Brown, H. T. and Escomb, F. Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Roy. Soc. Lond. Phil. Trans. B. **193**: 223–291. 1900.
28. Brücke, E. Das Verhalten der sog. Protoplasma-Ströme in den Brennhaaren von *Urtica urens* gegen die Schläge des Magnetelektromotors. Sitzungsber. Akad. Wiss. Wien. Math.-Nat. **46**: Abt. II: 35–38. 1862.
Buy du, H. G. See DuBuy.
29. Buller, A. H. R. Researches on fungi. Vol. 5. 1933.
30. Bungenberg, de Jong, H. G. Die Koazervation und ihre Bedeutung für die Biologie. Protoplasma **15**: 110–173. 1932.
31. Bünning, E. Die Physiologie des Wachstums und der Bewegungen. II. In Bünning, E. *et al.* Lehrbuch des Pflanzenphysiologie. 1939.
32. Burkholder, P. R. Movement in the Cyanophyceae. Quart. Rev. Biol. **9**: 438–459. 1934.
33. Bushee, G. L. Occurrence and rate of protoplasmic streaming. Bot. Gaz. **46**: 50–53. 1908.
34. Camp, W. G. A method of cultivating Myxomycete plasmodia. Bull. Torrey Bot. Club **63**: 205–210. 1936.
35. ————. The structure and activities of Myxomycete plasmodia. Bull. Torrey Bot. Club **64**: 307–335. 1937.
36. Castle, E. S. The influence of certain external factors on the spiral growth of single plant cells in relation to protoplasmic streaming. Jour. Cell. Comp. Physiol. **7**: 445–454. 1936.
37. Cazalas, M. Sur l'évolution du vacuome des *Chara* dans ses relations avec les mouvements du cytoplasme. Compt. Rend. Acad. Sci., Paris **190**: 314–317. 1930.
38. Chadefaud, M. Existence d'une structure infravisible orientée du cytoplasme chez lez Algues. Compt. Rend. Acad. Sci., Paris **196**: 423–424. 1933.
39. Cienkowski, L. Die Entwicklungsgeschichte der Myxomyceten. Jahrb. Wiss. Bot. **3**: 325–337. 1863.
40. ————. Das Plasmodium. Jahrb. Wiss. Bot. **3**: 400–441. 1863.
41. Clark, J. Über den Einfluss niederer Sauerstoffpressungen auf die Bewegungen des Protoplasmas. Ber. Deut. Bot. Ges. **6**: 273–280. 1888.
42. ————. On the protoplasmic movements and their relations to oxygen pressure. Diss. Tübingen. 1900.
43. Clifford, J. B. Notes on some physiological properties of a Myxomycete plasmodium. Ann. Bot. **11**: 179–186. 1897.
44. Colla, S. Nota sull 'azione della concentrazione degli idrogenioni sulle correnti protoplasmatische. Protoplasma **5**: 179–190. 1928.
45. ————. Untersuchungen über Plasma und Plasmaströmung bei Characeen. II. Die Wirkung verschiedener Salze auf die Plasmaströmung. Protoplasma **6**: 438–448. 1929.
46. Coman, D. R. Additional observations on positive and negative chemotaxis (Experiments with a myxomycete). Arch. Pathol. **29**: 220–228. 1940.
47. Cook, S. F. The effect of sudden changes of temperature on protoplasmic streaming. Jour. Gen. Physiol. **12**: 793–813. 1929.

48. Corti, B. Osservazioni microscopiche sulla *Tremella* e sulla circolazione del fluido in una Pianta acquajuola Lucca p. 127 et seq. 1774.
49. ———. Sur la circulation d'un fluide, découverte en diverses plantes. Rosier Observ. sur la Physique, sur l'Histoire nat. etc. **8**: 232 et seq. 1776.
50. Curtis, O. F. The translocation of solutes in plants. 1935.
51. DuBuy, H. G. Energy relations in transport. Proc. Univ. Md. Biol. Soc. Bull. Sch. Med. **24**: (4). 1940.
52. DuBuy, H. G. and Neurnbergk. E. Phototropismus und Wachstum der Pflanzen. III. Teil. 1935.
53. ——— and Olson, R. A. Protoplasmic streaming and dynamics of transport through living cells. Biodynamica **36**: 1–18. 1938.
54. ——— and ———. The relation between respiration, protoplasmic streaming and auxin transport in the *Avena* coleoptile, using a polarographic microrespirometer. Am. Jour. Bot. **27**: 401–413. 1940.
55. Dujardin, F. Recherches sur les organismes inférieures. Ann. Sci. Nat. II. Zool. **4**: 343–377. 1835.
56. Dutrochet, M. Observations sur la circulation des fluides chez la *Chara fragilis*. Ann. Sci. Nat. II. Bot. **9**: 5–38, 65–79. 1838.
57. Emoto, Y. Über die Chemotaxis der Myxomyceten-Plasmodien. Proc. Imp. Akad. Tokyo **8**: 460–463. 1932.
58. Emoto and Jochims. Arch. f. d. ges. Physiol. **216**: 611. 1927.
59. Ewart, A. J. On the physics and physiology of protoplasmic streaming in plants. 1903.
60. ———. The ascent of water in trees. Phil. Trans. Royal Soc. London B. **198**: 41–85. 1905.
61. Fenn, W. O. A new method for the simultaneous determination of minute amounts of carbon dioxide and oxygen. Am. Jour. Physiol. **84**: 110. 1928.
62. Fitting, H. Untersuchungen über die Auslösung von Protoplasmaströmung. Jahrb. Wiss. Bot. **64**: 281–388. 1925.
63. ———. Untersuchungen über Chemodinese bei *Vallisneria*. Jahrb. Wiss. Bot. **67**: 427–596. 1928.
64. ———. Über die Auslösung von Plasmaströmung durch optischaktive Aminosäuren. Jahrb. Wiss. Bot. **70**: 1–25. 1929.
65. ———. Untersuchungen über die Natur der chemodinetischen Reizung und über die Unterschiedsschwellen für *l*-Asparagin. Zeits. Bot. **23**: 328–360. 1930.
66. ———. Untersuchungen über endogene Chemonastie bei *Mimosa pudica*. Jahrb. Wiss. Bot. **72**: 700–775. 1930.
67. ———. Untersuchungen über die Empfindlichkeit und das Unterscheidungsvermögen der *Vallisneria* Protoplasten für verschiedene α-Aminosauren. Jahrb. Wiss. Bot. **77**: 1–103. 1932.
68. ———. Untersuchungen über den Plasmaströmung auslösenden Reizstoff in den Blattextrakten von *Vallisneria*. Jahrb. Wiss. Bot. **78**: 319–398. 1933.
69. ———. Untersuchungen über die Protoplasmaströmung auslösenden Reizstoff der Pflanzen. Naturwiss. **21**: 489–495. 1933.
70. ———. Über Auslösung von Protoplasmaströmung bei *Vallisneria* durch einige Histidin Verbindungen. Jahrb. Wiss. Bot. **82**: 613–624. 1936.
71. ———. Beiträge zur Physiologie der Protoplasmaströmung in den Blättern von *Vallisneria* spiralis. Ber. Deut. Bot. Ges. **55**: 255–261. 1937.
72. Freundlich, H. Kapillarchemie. 1930–32.
73. Gehenio, P. M. and Luyet, B. J. A study of the mechanism of death by cold in the plasmodium of the Myxomycetes. Biodynamica (55): 1–22. 1939.

74. Gelfan, S. A bioelectric potential. Science **67**: 589. 1928.
75. Gibbs, R. D. The action of ultraviolet light on *Spirogyra*. Trans. Roy. Soc. Canada **20** Sec. V: 419–426. 1926.
76. Goeppert, H. R. and Cohn, F. Über die Rotation des Zellinhaltes in *Nitella flexilis*. Bot. Zeit. **7**: 665–673, 681–691, 697–705, 713–719. 1849.
77. Grehn, J. Untersuchungen über Gestalt und Funktion der Sporangienträger bei den Mucorineen. II. Der Wasserund Stofftransport. Jahrb. Wiss. Bot. **76**: 167–207. 1932.
78. Guilliermond, A. The cytoplasm of the plant cell. 1941.
79. Hartig, T. Über die Bewegung des Saftes in den Holzpflanzen. Bot. Zeit. **16**: 329–335, 337–342. 1858.
80. Harvey, E. N. and Daniell, J. F. The elasticity of thin films in relation to the cell surface. Jour. Cell Comp. Physiol. **8**: 31–36. 1936.
81. Hauptfleisch, P. Untersuchungen über die Strömung des Protoplasmas in behäuteten Zellen. Jahrb. Wiss. Bot. **24**: 173–234. 1892.
82. Heidenhain, M. Plasma und Zelle. 1907–1911.
83. Heilbrunn, L. V. and Daugherty, K. The electric charge of protoplasmic colloids. Physiol. Zool. **12**: 1–12. 1939.
84. Hilton, A. E. On the cause of reversing currents in the plasmodia of Mycetozoa. Jour. Quekett Micr. Club II. **10**: 263–270. 1908.
85. Hill, S. E. Stimulation by cold in *Nitella*. Jour. Gen. Physiol. **18**: 357–367. 1934.
86. Höfler, K. Aus der Protoplasmatik der Diatomeen. Ber. Deut. Bot. Ges. **58**: 97–120. 1940.
87. Hofmeister, W. Die Lehre von der Pflanzenzelle. 1867.
88. Honert, T. H. van den. On the mechanism of the transport of organic materials in plants. Proc. Kon. Akad. Wet. Amsterdam **35**: 1104– 1932.
89. Hörmann, G. Studien über die Protoplasmaströmung bei den Characeen. 1898.
90. Hosoi, T. Protoplasmic streaming in isolated pieces of *Paramecium*. Jour. Fac. Sci. Imp. Univ. Tokyo. Sect. IV. **4**: 299–305. 1937.
91. Howard, F. L. The laboratory cultivation of Myxomycete plasmodia. Am. Jour. Bot. **18**: 624–628. 1931.
92. Jahn, E. Die Organe des Plasmodiums. Ber. Deut. Bot. Ges. **50a**: 369–399. 1932.
93. Jöhnsson, B. Der richtende Einfluss strömenden Wassers auf wachsende Pflanzen und Pflanzentheile (Rheotropism). Ber. Deut. Bot. Ges. **1**: 512–521. 1883.
94. Jost, I. Einige physikalische Eigenschaften des Protoplasmas von *Valonia* und *Chara*. Protoplasma **7**: 1–21. 1929.
95. Jurišić, P. J. Osmotischer Druck und Protoplasmaströmung in Pflanzenzellen. Zeits. Allg. Physiol. **20**: 100– . 1922.
96. ———. Die Geschwindlichkeitsänderung der Protoplasmaströmung in den Pflanzenzellen. Act. Bot. Inst. R. Univ. Zagreb. **1**: 25– . 1925.
97. Jungers, V. Die Verlagerungsfähigkeit des Zellinhaltes der Zweibelschuppen von *Allium cepa* durch Zentrifugierung. Protoplasma **21**: 351–361. 1934.
98. Kamitz, A. Temperatur und Lebensvorgänge. 1915.
99. Kamiya, N. Die Rhythmik des metabolischen Formwechsels der Euglenen. Ber. Deut. Bot. Ges. **57**: 231–240. 1939.
100. ———. The control of protoplasmic streaming. Science **92**: 462–463. 1940.
101. ? ———. Physical aspects of protoplasmic streaming. In Structure of Protoplasm. 199–244. 1942.
102. Keferstein, M. H. Untersuchungen über die Rheotaxis der Plasmodien. Bot. Arch. **20**: 1–21. 1927.

103. Kinoshita, S. Über die Wirkung des Wuchsstoffes auf die Bewegung des Plasmodiums. Bot. Mag. (Tokyo) **52**: 492–497. 1938.

104. ———. Über den Einfluss des Wuchsstoffes auf die Protoplasmaströmung bei den Myxomyceten-Plasmodien. Bot. Mag. (Tokyo) **53**: 175–180. 1939.

105. ———. Über den Einfluss des elektrischen Stromes auf die Rhythmik der Protoplasmaströmung bei den Myxomyceten-Plasmodien. Bot. Mag. (Tokyo) **54**: 52–57, 1940.

106. Kirchheimer, F. Protoplasma und Wundheilung bei *Phycomyces*. Planta **19**: 574–606. 1933.

107. Kitching, J. A. and Pirenne, M. H. The influence of low tensions of oxygen on the protoplasmic streaming of Myxomycetes. Jour. Comp. Cell. Physiol. **16**: 131–133. 1940.

108. Klebs, G. Über Form und Wesen der pflanzlichen Protoplasmabewegung. Biol. Centralbl. **1**: 513–524. 1881–82.

109. ———. Über die Organisation einiger Flagellatengruppen und ihre Beziehungen zu Algen und Infusorien. Unters. Tübingen **1**: 233–362. 1883.

110. Kôketsu, R. Über die Wirkungen der elektrischen Reizung auf den pflanzlichen Zellgebilden. Jour. Dept. Agr. Kyushu Imp. Univ. **1**: 1–133. 1923.

111. Kühne, W. Untersuchungen über das Protoplasma und die Contractilität. 1864.

112. Küster, E. Über Inhaltsverlagerungen in plasmolysierten Zellen. Flora **100**: 267–287. 1910.

113. ———. Protoplasmabewegungen in zentrifugierten Zellen. Ber. Oberhess. Ges. Nat. u. Helik (Giessen) **15**: 194–218. 1932.

114. ———. Die Pflanzenzelle. 1935.

115. Lambers, M. H. R. The influence of temperature on protoplasmic streaming of Characeae. Proc. Kon. Akad. Wet. Amsterdam **28**: 340–346. 1925.

116. ———. Temperatur en protoplasmastrooming Diss. Utrecht, Amsterdam. 1926.

117. Lauterbach, L. Untersuchungen über die Beeinflussung der Protoplasmaströmung der Characeen durch mechanische und osmotische Eingriffe. Beih. Bot. Centralbl. **38**: I Abt. 1–52. 1921.

118. Leblond, E. Electrophorese colloidale et movement protoplasmiques. Compt. Rend. Soc. Biol. **98**: 580– . 1928.

119. Lepow, S. S. Some reactions of slime mold protoplasm in certain alkaloids and snake venoms. Protoplasma **31**: 161–179. 1938.

120. Lewis, W. H. On the role of a superficial plasmagel layer in division, locomotion and changes in form of cells. Arch. Exp. Zellf. **22**: 270– . 1938.

121.? ———. The relation of the viscosity changes of protoplasm to amoeboid locomotion and cell division. Structure of Protoplasm 163–197. 1942. Ames, Iowa.

122. Macht, D. and Lubin, D. S. A phytopharmacological study of menstrual toxin. Jour. Pharm. & Exp. Therap. **22**: 413– . 1923.

123. Marcy, B. Effect of ethylene chlorhydrin and thiourea on *Elodea* and *Nitella*. Plant Physiol. **12**: 207–212. 1937.

124. Marsland, D. A. The mechanism of protoplasmic streaming. The effect of high hydrostatic pressure upon cyclosis in *Elodea canadensis*. Jour. Cell Comp. Physiol. **13**: 23–30. 1939.

125. ———. Protoplasmic streaming in relation to gel structure in the cytoplasm. Pp. 127–161 in The Structure of Protoplasm, Ames, Iowa, 1942.

126. Marsland, D. A. and Brown, D. E. S. Amoeboid movement at high hydrostatic pressure. Jour. Cell. Comp. Physiol. **8**: 167–178. 1936.

127. Mason, F. G. and Maskell, E. J. Studies on the transport of carbo-

hydrates in the cotton plant. I, II. Ann. Bot. **42**: 189–253, 571–636. 1928.
128. Mast, S. O. Locomotion in *Amoeba proteus* (Leiby). Protoplasma **14**: 321–330. 1931.
129. Meyen, F. J. F. Über die eigenthümliche Saftbewegung in den Zellen der Pflanzen. Nova. Acta. Acad. Caes. Leo. Carol. **13**: Nat. Cur. 839– . 1827.
130. ———. Neues System der Pflanzenphysiologie. Vol. 2: 206–258. 1838.
131. Meyer, A. Die Plasmabewegung verursacht durch eine geordnete Wärmebewegung von Molekülen. Ber. Deut. Bot. Ges. **38**: 36–43. 1920.
132. ———. Morphologische und physiologische Analyse der Zelle der Pflanzen und Tiere. 2 Teil. 1926.
133. Mohl, H. v. Über die Saftbewegung im Inneren der Zelle. Bot. Ztg. **4**: 73–78, 89–94. 1846.
134. Naegeli, C. v. Zellkerne, Zellbildung und Zellwachsthum. Zeits. Wiss. Bot. *I*: pt. 1: 34–127; pt. 3–4: 22–92. 1844.
135. ———. Gattungen einzelliger Algen. 1849.
136. Noethling, W. und Rochlin, E. Über Photodinese im kurzwelligen Ultraviolett. Planta **14**: 112–131. 1931.
137. Northern, H. T. Studies of protoplasmic structure in *Spirogyra*. I. Elasticity. Protoplasma **31**: 1–8. 1938.
138. Nothmann-Zuckerhandl, H. Die Wirkung der Narkotika auf die Plasmaströmung. Biochem. Zeits. **45**: 412–451. 1912.
139. ———. Über die Erregung der Protoplasmaströmung durch verschiedene Strahlenarten. Ber. Deut. Bot. Ges. **33**: 301–313. 1915.
140. Olson, R. A. and DuBuy, H. G. Factors influencing the protoplasmic streaming in oat coleoptile. Am. Jour. Bot. **27**: 392–401. 1940.
141. Oort, A. J. P. and Roelofsen, P. A. Spiralwachstum, Wandbau und Protoplasmaströmung bei *Phycomyces*. Proc. Royal Acad. Wet. Amsterdam **35**: 898–909. 1932.
142. Pascher, A. Von der merkwürdigen Bewegungsweise einiger Flagellaten. Biol. Zentralbl. **37**: 421–429. 1917.
143. Péterfi, T. und Yamaha, G. Die Wirkung des mechanischen Drucks auf das Protoplasma der *Nitella*-Zelle. Protoplasma **12**: 279–286. 1931.
144. Pfeffer, W. The physiology of plants. English ed. 1906.
145. Pfeiffer, H. H. Experimentelle Cytologie. 1940.
146. Planthaber, H. Über den Einfluss von Giften auf Protoplasmaströmung. Diss. Kiel. 1908.
147. Plowe, J. Q. Membranes in the plant cell. I. Morphological membranes at protoplasmic surfaces; II. Localization of differential permeability in the plant protoplast. Protoplasma **12**: 196–240. 1931.
148. Pop, L. J. J. Protoplasmic streaming in relation to spiral growth of *Phycomyces*. Kon. Akad. Wet. Amsterdam **41**: 661–672. 1938.
149. Prell, H. Die Bewegungen der Cyanophyceen. Arch. Protistenk. **42**: 99–156. 1921.
150. Pringsheim, N. Über Lichtwirkung und Chlorophyllfunktion in der Pflanze. Jahrb. Wiss. Bot. **12**: 288–437. 1882.
151. Rashevsky, N. Mathematical biophysics of the cell with reference to the contractility of tissues and amoeboid movements. Bull. Math. Biophys. **1**: 47–62. 1939.
152. Reichert, C. B. Über die Saftströmung (Rotation, Circulation der Pflanzenzellen) mit Rücksicht auf die Contratilitätsfrage. Monatsber. Kon. Preuss. Akad. Wiss. Berlin 318– . 1866.
153. Robbins, W. J. The isoelectric point for plant tissue and its importance in absorption and toxicity. Univ. Missouri Studies **1**: 3–60. 1926.
154. Romijin, G. Über den Einfluss der Temperature auf die Protoplasma-

strömung bei *Nitella flexilis.* Kon. Akad. Wet. Amsterdam **34**: 289–296. 1931.
155. Rosanoff, S. De l'influence de l'attraction terrestre sur la direction des plasmodia des Myxomycetes. Mem. Soc. Sci. Nat. Cherbourg **19**: 149–172. 1868.
156. Samassa, P. Über die Einwirkung von Gasen auf die Protoplasmaströmung und Zellteilung von *Tradescantia* sowie auch die Embryonalentwicklung von *Rana* und *Ascaris.* Verh. Nat. Med. Ver. Heidelberg N. F. **6**: 1–16. 1898.
157. Scarth, G. W. The structural organization of plant protoplasm in the light of micrurgy. Protoplasma **2**: 189–205. 1927.
158. ————. Structural differentiation of cytoplasm, in, The Structure of Protoplasm, Ames, Iowa, pp. 99–107. 1942.
159. Schorr, L. Anthozyan im Protoplasma. Beiträge zur Zellenphysiologischen Methodik. Zts. f. Wiss. Mikr. **52**: 369–379. 1935–36.
160. Schleiden, M. Principles of scientific botany (Eng. trans. by Edwin Lankester). 1849.
161. Schneider, C. L. The interdependence of auxin and sugar for growth. Am. Jour. Bot. **25**: 258–270. 1938.
162. Schröter, A. Über Protoplasmaströmung bei Mucorineen. Flora **95**: 1–30. 1905.
163. Schultze, M. S. Über den Organismus der Polythalamien nebst Bemerkungen über die Rhizopoden in den Allgemeinen. 1854.
164. ————. Über Muskelkörperchen und das was man eine Zelle zu nennen habe. Arch. Anat. Physiol. 1–27. 1861.
165. ————. Das Protoplasma der Rhizopoden und der Pflanzenzellen. 1863.
166. Schulze, J. Über die Einwirkung der Lichtstrahlen von 280 $\mu\mu$ Wellenlänge auf Pflanzenzellen. Beih. Bot. Centralbl. **25** Abt. I: 30–79. 1910.
167. Schuster, G. Über den Einfluss der Sauerstoffpressung auf die Protoplasmaströmung. Diss. Leipzig. 1913.
168. Schwann, T. Microscopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachstum der Tiere und Pflanzen. 1839.
169. Schweickerdt, H. Untersuchungen über Photodinese bei *Vallisneria spiralis.* Jahrb. Wiss. Bot. **68**: 79–134. 1928.
170. Seifriz, W. A method for inducing protoplasmic streaming. New Phyt. **21**: 107–112. 1922.
171. ————. Elasticity and some structural features of soap solutions. Colloid Symposium Monograph **3**: 285–295. 1925.
172. ————. Reaction of protoplasm to radium radiation. Protoplasma **25**: 196–200. 1936.
173. ————. Protoplasm. 1936.
174. ————. A theory of protoplasmic streaming. Science **86**: 397–398. 1937.
175. ————. A materialistic interpretation of life. Philosophy of Science **6**: 266–284. 1939.
176. ————. Pathological changes in protoplasm. Protoplasma **32**: 538–550. 1939.
177. ————. A theory of anesthesia based on protoplasmic behavior. Anesthesiology **2**: 300–309. 1941.
178. ————. Some physical properties of protoplasm and their bearing on structure: in, The Structure of Protoplasm, Ames, Iowa, 245–264. 1942.
179. ———— and Epstein, N. Shock anesthesia in Myxomycetes. Biodynamica **3** (67): 191–197. 1941.
180. ———— and Russell, M. A. The fruiting of Myxomycetes. New Phyt. **35**: 472–478. 1936.

181. ———— AND URAGUCHI, M. The toxic effects of heavy metals on protoplasm. Am. Jour. Bot. **28**: 191–197. 1941.
182. SPEK, J. Oberflächenspannungsdifferenzen als eine Ursache der Zellteilung. Arch. Entw. Mech. **44**: 1–113, 217–225. 1918.
183. SSAWOSTIN, P. W. Magnetophysiologische Untersuchungen. I. Die Rotationsbewegung des Plasmas in einem konstanten magnetischen Kraftfelde. Planta **11**: 683–726. 1930.
184. STAHL, E. Zur Biologie der Myxomyceten. Bot. Ztg. **42**: 145–156, 161–176, 187–191. 1884.
185. STRASBURGER, E. Wirkung des Lichtes und Wärme auf die Schwärmsporen. 1878.
186. ————. Über den Bau und die Verdichtungen der Leitungsbahnen in den Pflanzen. 1891.
187. ————. Über Cytoplasmastrukturen, Kern- und Zellteilung. Jahrb. Wiss. Bot. **30**: 375–405. 1897.
188. STRUGGER, S. Untersuchungen über den Einfluss der Wasserstoffionen auf das Protoplasma der Wurzelhaare von *Hordeum vulgare* L. I. Sitzungsber. Akad. Wiss. Wien, Math.-Nat. Abt. I. **135**: 453–478. 1926.
189. ————. Untersuchungen über den Einfluss der Wasserstoffionen auf das Protoplasma der Wurzelhaare von *Hordeum vulgare* L. II. Sitzungsber. Akad. Wiss. Wien, Math.-Nat. Abt. I. **137**: 143–169. 1928.
190. SWEENEY, B. M. AND THIMANN, K. V. The effect of auxins on protoplasmic streaming. II. Jour. Gen. Physiol. **21**: 439–461. 1938.
191. TERNETZ, C. Protoplasmabewegung und Truchtkörperbildung bei *Ascophanus carneus* Pers. Jahrb. Wiss. Bot. **35**: 273–312. 1900.
192. THIMANN, K. V. Growth substances in plants. Ann. Rev. Biochem. **4**: 545–568. 1935.
193. ———— AND SCHNEIDER, C. L. The relative activities of different auxins. Am. Jour. Bot. **26**: 328–333. 1939.
194. ———— AND SWEENEY, B. M. Action of auxin on protoplasmic streaming. Nature **140**: 807–808. 1937.
195. ———— AND ————. The effect of auxins upon protoplasmic streaming. Jour. Gen. Physiol. **21**: 123–135. 1937.
196. TIEGS, O. W. Surface tension and theory of protoplasmic movement. Protoplasma **4**: 88–139. 1928.
197. TREVIRANUS, L. C. Beobachtungen über die Bewegung des körnigen Wesens in einigen Conferven und einer Chara. *In* Weber, F. Beiträge zur Naturkunde Vol. **2**: 132. 1810.
198. ————. Beytrage zur Pflanzenphysiologie. 1811.
199. VALKANOV, A. Über die kinetische Energie einiger Zellbestandteile, Protoplasma **20**: 20–30. 1934.
200. VELTEN, W. Über die Verbreitung der Protoplasmabewegungen im Pflanzenreiche. Bot. Ztg. **30**: 645–653. 1872.
201. ————. Bewegung und Bau des Protoplasmas. Flora **56**: 81–89, 97–102, 113–128. 1873.
202. ————. Einwirkung strömender Elektrizität auf die Bewegung des Protoplasmas, auf den lebendigen und todten Zelleninhalt, sowie auf materielle Theilchen überhaupt. Sitzungsber. Akad. Wiss. Wien. Math.-Nat. **73** Abt. I: 343–376. 1876.
203. ————. Die Einwirkung der Temperatur auf die Protoplasmaströmung. Flora **59**: 177–182, 193–199, 209–217. 1876.
204. VERWORN, M. Die Bewegungen der lebendigen Substanz. Untersuchung der Kontraktionserscheinungen. 1892.
205. ————. General physiology. 1899.
206. VESQUE-PÜTTLINGEN, F. v. Notiz über Periodizität der Protoplasmaströmung. Bot. Ztg. **34**: 572–575. 1876.
207. VINES, S. H. Lectures on the physiology of plants. 1886.

208. Voronin, M. Beiträge zur Morphologie und Physiologie der Pilze. II. Entwicklungsgeschichte des *Ascophanus pulcherrimus* Cr. 1866.
209. Vouk, V. Untersuchungen über die Bewegungen der Plasmodien. I. Die Rhythmik der Protoplasmaströmung. Sitzungsber. Akad. Wiss. Wien. Math.-Nat. **119**: 853–876. 1910.
210. ———. Untersuchungen über die Bewegungen der Plasmodien. II. Studien über die Protoplasmaströmung. Denkschr. Akad. Wiss. Wien. Math.-Nat. **88**: 652–692. 1913.
211. Vries, H. de. Über die Bedeutung der Circulation und der Rotation des Protoplasmas für den Stofftransport in der Pflanze. Bot. Ztg. **43**: 1–6, 17–26. 1885.
212. Watanabe, A., Kodati, M. and Kinoshita, S. Über die Beziehung zwischen der Protoplasmaströmung und der elektrischen Potentialveränderungen bei Myxomyceten. Bot. Mag. (Tokyo) **51**: 337–349. 1937.
213. ———. Über die negativen Galvanotaxis der Myxomyceten-Plasmodien. Bot. Mag. (Tokyo) **52**: 441–445. 1938.
214. ———. Über den Einfluss von verschiedenen Substanzen auf das bioelektrische Potential der Myxomyceten-Plasmodien. Bot. Mag. (Tokyo) **52**: 598–607. 1938.
215. ———. Über den Einfluss des Wuchsstoffes auf das bioelektrische Potential der Myxomyceten-Plasmodiem. Bot. Mag. (Tokyo) **53**: 32–42. 1939.
216. ———. Über die Potentialdifferenzen zwischen den Myxomyceten-Plasmodien und den Aussenmedien. Bot. Mag. (Tokyo) **53**: 410–414. 1939.
217. ———. Über die Einflüsse des Wuchsstoffes und der anderen Substanzen auf die Potentialdifferenzen zwischen dem Plasmodium und dem Aussenmedium. Bot. Mag. (Tokyo) **53**: 460–465. 1939.
218. Went, F. W. Remarks about two auxin problems. Chron. Bot. **4**: 503–505. 1938.
219. Williams, M. Observations on the action of X-rays on plant cells. Ann. Bot. **37**: 217–223. 1923.
220. ———. Some observations on the action of radium on certain plant cells. Ann. Bot. **39**: 547–562. 1925.
221. Winer, B. J. and Moore, A. R. Reactions of the plasmodium *Physarum polycephalum* to physico-chemical changes in the environment. Biodynamica 3 (73): 323–345. 1941.
222. Wood, R. W. Supersonics. 1939.
Woronin, M. See Voronin.
223. Yagi, Ultrasonics and organisms. Bot. & Zool. **8**: 1312–1314. 1940.
224. Yamaha, G. Recent protoplasmology. Bot. & Zool. **8**: 939–945. 1940.
225. Yamaha, G. and M. Ida. Protoplasmaströmung (Literatur). Protoplasma **17**: 146–158. 1932.

Anthocyanin Pigments	F. Blank *Switzerland*
The Genetics of Pollen Tube Growth	J. T. Buchholz *University of Illinois*
The Inferior Ovary	Gertrude E. Douglas *New York State College for Teachers*
The Angiosperm Embryo Sac	Emma L. Fisk *University of Wisconsin*
Protoplasmic Connections	L. G. Livingston *Harvard University*
Tropical Fruits and Their Future	S. J. Lynch *Sub-tropical Experiment Station*
Cell-shape	E. B. Matzke *Columbia University*
Recent Advances in the Science and Technique of Seed Analysis	R. H. Porter *Iowa State College*
Polyploidy	L. F. Randolph *Cornell University*
Distribution and Composition of Paleozoic Floras in North America	C. R. Read *U. S. Geological Survey*
Bacterial Galls	A. J. Riker *University of Wisconsin*
Phylogeny of the Basidiomycetes	D. P. Rogers *Brown University*
Cell Physiology	G. W. Scarth *McGill University*
Botanical Contributions to the Study of Coal	J. M. Schöpf *Illinois State Geological Survey*
Recent Studies on the Inheritance of Size and Shape in Plants	C. W. Sharsmith *University of Minnesota*
History of Arctic and Alpine Floras	H. H. Smith *Arlington Experiment Farm*
Measurement of Soil Moisture	F. J. Veihmeyer *University of California*
Utilization of Native Plants by the American Indians	P. A. Vestal *Harvard University*
The St. Lawrence River as a Biological Habitat for Higher Plants	Fr. Marie-Victorin *University of Montreal*
Effect of Light on Respiration	R. L. Weintraub *Smithsonian Institute*
Heterosis	W. G. Whaley *Barnard College*
Control of Noxious Plants	H. K. Wilson *University of Minnesota*
Plant Microfossils	L. R. Wilson *Coe College*
Modern Views and Facts About Growth Substances	P. W. Zimmerman *Boyer Thompson Institute for Plant Research*

Proposed Future Contents of
THE BOTANICAL REVIEW

Articles received and awaiting publication

Breeding for Disease Resistance in Wheat, Oats, Barley and Flax	E. R. AUSEMUS, *Bureau of Plant Industry*
Market Pathology	C. O. BRATLEY
Polarity in Plants	ROBERT BLOCH, *Yale University*
Origin and Development of Primary Vascular Tissues in Seed Plants	KATHERINE ESAU, *University of California*
Metabolic Phenomena in Plants Associated with Virus Infection	F. L. WYND, *University of Illinois*
Effects of Fire on Vegetation of the Southeastern United States	KENNETH H. GARREN, *Georgia Agricultural Experiment Station*
Vegetational Zonation in the Rocky Mountains	R. F. DAUBENMIRE, *University of Idaho*
Geographical Distribution of Fungi	G. R. BISBY, *Imperial Mycological Institute, Kew*
Osmosis and Osmotic Pressure	H. C. EYSTER, *University of South Dakota*
Plant Life and the Law of Man. IV. Barberry, Currant and Gooseberry, and Cedar Control	E. H. FULLING, *The Botanical Review*
The Cuticle in Angiosperms	J. H. PRIESTLEY, *University of Leeds*
Lichens—Their Biological and Economic Significance	G. A. PEREZ-LLANO, *Harvard University*
Root-rots of Some Non-cereal Crops	G. H. BERKELEY, *Dominion Laboratory of Plant Pathology*

Articles arranged for most recently

Genetics of Neurospora	C. L. LINDEGREN, *Washington University*
Sexuality and Genetics of Algae	H. BOLD and W. G. WHALEY, *Columbia University*
A Critical Survey of the Present Status of Plant Embryology	D. A. JOHANSEN, *Stanford University*
Air Space Tissue in Plants	H. B. SIFTON, *University of Toronto*
Phytogeography of Patagonia	A. A. BEETLE, *University of California*
Phytogeography of the Temperate West Coast of South America	H. E. STORK, *Carleton College* and J. L. MORRISON, *University of California*

Articles in course of preparation

The Cytology of Fertilization in Angiosperms	L. E. ANDERSON, *Duke University*
Relation of Wood Anatomy to Taxonomy	I. W. BAILEY, *Harvard University*

(*Continued inside*)